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*Abies lasiocarpa*



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# Phytologia

## Contents

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R. P. Adams. Intraspecific terpenoid variation in <i>Juniperus scopulorum</i> Pleistocene refugia and Post-Pleistocene recolonization.....	3
J. E. Ebinger, D. S. Seigler and L. R. Phillipe. Understory vegetation of thorn scrub woodlands at the Chaparral wildlife management area, Dimmit and LaSalle Counties, Texas.....	13
R. P. Adams and D. Thornburg. Sexual change in <i>Juniperus arizonica</i> : facultative monecious?.....	43
R. P. Adams. Chemosystematics of <i>Juniperus</i> : Effects of leaf drying on essential oil composition: II.....	51
D. B Ward. Keys to the flora of Florida - 27. <i>Fraxinus</i> (Oleaceae)...	63
R. P. Adams, C. Earle and D. Thornburg. Taxonomy of infraspecific taxa of <i>Abies lasiocarpa</i> : Leaf essential oils and DNA of <i>Abies lasiocarpa</i> , var. <i>bifolia</i> , var. <i>arizonica</i> .....	73
B. L. Turner. A new gypsophilic <i>Phacelia</i> (Hydrophyllaceae) from Coahuila, Mexico.....	88
H. Robinson. <i>Ageratina tovarae</i> , a new species from northern Peru (Asteraceae: Eupatorieae).....	94
B. L. Turner. Routine identification of Mexican plants has occasioned the recension of <i>Malacomeles</i> (Rosaceae).....	99
Cover Photo. <i>Abies lasiocarpa</i> Photo by Christopher J. Earle, see Adams, Earle and Thornberg, p. 73.	



- R. P. Adams, R. Lanner, M. Kauffmann and D. Thornburg.  
Intraspecific variation in the leaf essential oils of *Abies concolor*.....107
- R. P. Adams, C-F. Hsieh, J. Murata and A. E. Schwarzbach.  
Systematics of *Juniperus chinensis* and *J. tsukusiensis* from Japan and  
Taiwan: DNA sequencing and terpenoids.....118
- R. P. Adams. The taxonomic affinity of a juniper population from  
Colonia Pacheco, Mexico .....132
- R. P. Adams and A. E. Schwarzbach. DNA Barcoding a juniper: The  
case of the south Texas Duval County juniper and serrate junipers of  
North America.....146

## INFRASPECIFIC TERPENOID VARIATION IN *JUNIPERUS SCOPULORUM*: PLEISTOCENE REFUGIA AND POST-PLEISTOCENE RECOLONIZATION

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### ABSTRACT

The patterns of leaf terpenoid variation are examined from throughout the range of *Juniperus scopulorum*. The oils of all of the central Rocky Mountain populations are very uniform, suggesting that these populations were mostly displaced to lower elevation sites during the Pleistocene. Populations in Wallowa, Ore. and British Columbia (BC) (northern Rocky Mountains) are differentiated from the central Rocky Mountain populations. It is postulated that a refugium for this germplasm was in the Wallowa Mtns., Ore. and that the glaciated populations of BC were re-colonized by seeds from the Wallowa refugium. *Phytologia* 93(1): 3-12 (April 1, 2011).

**KEY WORDS:** *Juniperus scopulorum*, *J. blancoi*, *J. maritima*, *J. virginiana*, leaf terpenoids, geographic variation, Pleistocene refugia, recolonization, Wisconsin glaciation.

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In 1983, I published an analysis of geographic variation in leaf oils of *J. scopulorum* (Adams, 1983). It was reported that the samples from Puget Sound and Vancouver Island were the most distinct of all populations and that their oils were actually more similar to *J. virginiana* than *J. scopulorum* (Fig. 13, Adams, 1983). Subsequently, Adams (2007) recognized the Puget Sound and Vancouver Island (including the Strait of Georgia) plants as *J. maritima* R. P. Adams based on the combined use of terpenoids, morphology and nrDNA sequence data. In addition, analyses of nrDNA from herbarium specimens of the juniper from Serranias del Burro, Mexico (Adams, in prep.) indicate that the latter is a relictual population of *J. virginiana*,

not *J. scopulorum* as treated in Adams (1983, 2008). The inclusion of these two species (*J. maritima*, *J. virginiana*) in the computation of ANOVA and SNK (Adams, 1983) biased the character weighting (F-1 weights). With this new knowledge of speciation in *Juniperus* from DNA sequencing, it is appropriate to re-examine variation in *J. scopulorum* with the aforementioned taxa excluded from ANOVA and SNK analyses.

## MATERIALS AND METHODS

Plant material: (species, population acronym, location, vouchers): *J. blancoi*: BO, El Oro, Mexico, 14 mi S of El Oro, 4.5 mi S of Carmona, 8460', *Adams 1486-1500*; BS, El Salto, Mexico, 3 mi S of El Salto on road to Guadalupe along stream, ca 8500', *Adams 1455, Zanoni 2766-2775*; *J. maritima*: PW, Bayview St. Park and Whidbey Island, WA, 3-100', *Adams 1740-1747*; VB, Vancouver Island, BC, Mill Bay and Cowichan Bay, seaside, 3-10', *Adams 2465-2477*; *J. scopulorum*: SI, 1 mi W of Soda Springs, ID on US30, 5779', *Adams 1662-1676*; TU, Thistle Utah, 5250', *Adams 1677-1688*; LN, 5.7 mi E of Lamoille, NV, 7500', *Adams 1708-1722*; WO, 3 mi W. of Wallowa, Ore., 2950', *Adams 1725-1739*; MB, Manning Park, BC, on Talus slope, on Canada Hwy 3, 7.4 mi E of Manning Park Lodge, 3500', *Adams 1749-1763*; TB, 3.6 mi E of Telkwa, BC on BC Hwy 16, 2100', *Adams 1765-1779*; WB, Williams Lake, BC, 3.7 mi S of William Lake on BC Hwy 97, 2100', *Adams 1780-1794*; DC, Dutch Creek, BC, 3.5 mi S of Fairmont, 2600', *Adams 1811-1825*; KM, Kalispell, MT, 4.5 mi. S of US 83 & MT 208 jct. on US 83, N of Lakeside, 2960', *Adams 1826-1840*; BM, Butte, MT, 3-4 mi. W of Butte, on I90, 5700', *Adams 1841-1855*; BR, Bridger, MT, 16.6 mi. N of Bridger on US310, 4100', with *J. osteosperma* and *J. horizontalis*, *Adams 1864-1877*; MM, Missouri River, MT, 3.4 mi. S of Missouri River Bridge on US191, 2700', *Adams 1881-1896*; AN, Amidon, ND, Burning Coal Seam Park, 12 mi NW of Amidon, 3200', *Adams 1902-1919*; NW, Newcastle, WY, on US 85, 0.9 mi N of jct of US 85 & WY 16, 4310', *Adams 1920-1934*; SC, Stove Prairie Landing, Poudre River, 15.8 mi. W of jct. US 287 and CO 14, 18 mi. W of Ft. Collins, CO, *Adams 1935-1949*; RN, Raton, NM, 2 mi. N of Raton, NM on I25, 6800', *Adams 1965-1979*; DC, Durango, CO, w of town on hill, 6600', *Adams 2010-2024*; NA, Nutrioso, AZ, on US 180, 2 mi. N of Nutrioso, 7900', *Adams 2052-*



2066; OA, Oak Creek Canyon, AZ, 16.2 mi. N of Sedona, on AZ 89A, 6500', *Adams 2097-2111*; CN, Charleston Peak, NV, at Ranger Station, 20 mi W of US 85 on NV 39, 7400', *Adams 2154-2167*; CU, Cedar City, UT, 7.5 mi E of Cedar City on UT 14, 6900', *Adams 2173-2187*; RD, Ruidoso, NM, between Ruidoso and NM 24 on US 70, 6600', *Adams 2233-2246*; CM, Colonia Pacheco, Mexico, 3 mi. E of Colonia Pacheco on Rio Piedras Verde, 6950', *Adams 2501-2515*; *J. virginiana/scopulorum*: PT, Palo Duro Canyon, TX, 1 mi E of Tanglewood Lake dam on the John Currie Ranch in deep canyons along the Prairie Dog Fork of the Red River, ca. 3300', *Adams 2263-2277*; *J. virginiana*: AO, Altus, OK, Quartz Mtn. St. Park, Lake Altus, OK, *Adams 2323-2337*; WD, Washington, DC, 10 mi. E of Dulles Airport, *Adams 2409-2423*; SM, Serranias del Burro, Mexico, Arroyo de la Zorro Canyon, ~6000', *Adams 2424-2438*; Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU). The distribution of *J. scopulorum* (*sensu stricto*) and populations sampled are shown in figure 1.

Isolation of Oils - See Adams (1983). Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by ANOVA and SNK (Student-Newman-Keuls) analyses (after Steele and Torrie, 1960). Gower or Manhattan metric (Adams, 1975; Gower, 1971) were computed among all populations using character weighting of F-1 (F from ANOVA). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

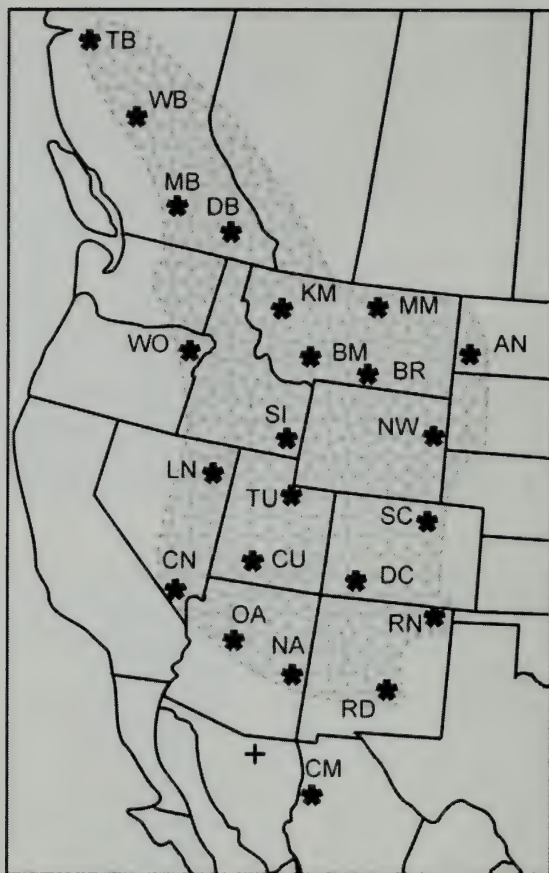


Figure 1. Distribution of *J. scopulorum* (*sensu stricto*) and populations sampled in this study. The + symbol in Sonora, Mexico is an outlier population.

## RESULTS AND DISCUSSION

SNK analyses of 177 leaf terpenoids of *J. scopulorum* revealed 33 compounds with F ratios (from ANOVA) and the largest population value greater than 0.5% concentration. The similarity matrix factored by PCO (Principal Coordinate Analysis). Nine

eigenroots accounted for 80% of the variance among populations. The eigenroots appeared to asymptote after the first five: 30.18, 15.57, 8.32, 6.43, 5.20%. Contoured similarities are shown in figure 2. The

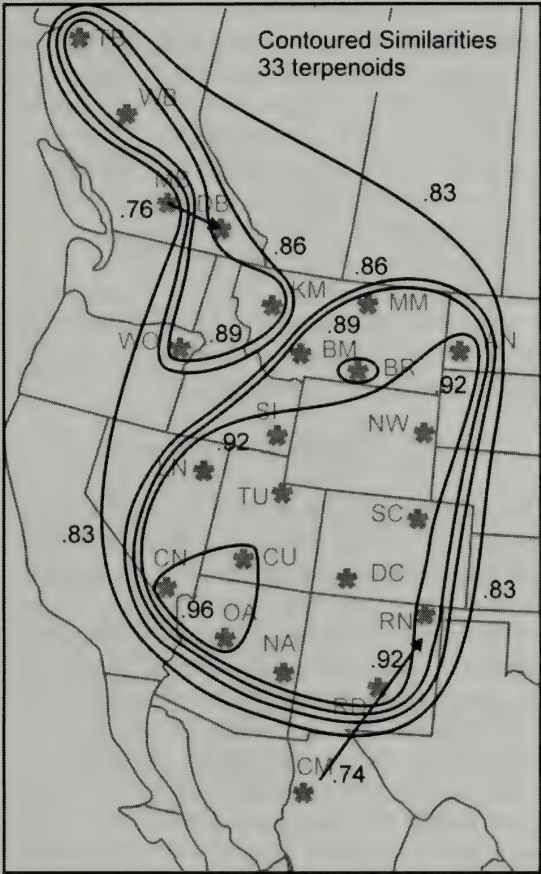


Figure 2. Contoured similarities among populations of *J. scopulorum* based on 33 terpenoids. See text for discussion.

oils from plants at Oak Creek, AZ (OA), Charleston Peak (CN) and Cedar City, UT (CU) were nearly identical (0.96 similarity, Fig. 2). The oils of all of the central Rocky Mountain populations were found to be very similar (0.89-0.96, Fig. 2). However, the populations from

the north-western Rocky Mountains form a separate group (Fig. 2, WO, KM, DB, WB, TB). The break between the two groups is quite distinct between Kalispell, MT (KM) and Butte, MT (Fig. 2).

The Colonia Pacheco, Mexico (CM) population was an outlier and had its highest similarity to the oil from Raton, NM (RN) with a similarity of 0.74 (Fig. 2). Because *J. blancoi* var. *mucronata* (R. P. Adams) Farjon grows near Yecora (about 140 air miles southwest of Colonia Pacheco), it is possible that the CM plants contain germplasm from *J. b. var. mucronata*. This question is beyond the scope of this paper and is currently being investigated (Adams, in prep.).

The oil of the Manning Park, BC population (MB) is another anomalous population that is joined to the nearby Dutch Creek, BC population (DB) by similarity of 0.76 (Fig. 2). There seems no apparent explanation for this divergence.

### Pleistocene Patterns

The late Wisconsin maximum ice advance is shown in figure 3 (based on Flint, 1971 and Crandell, 1971). All of the Canadian *J. scopulorum* populations were glaciated. In addition, the Kalispell (KM), Missouri River (MM) and Amidon, ND (AN) populations were probably exterminated. Other populations (BM, BR and NW) were likely displaced by boreal forests and tundra (Flint, 1971; Porter, 1971). *Juniperus scopulorum* is a lower montane species, with the widespread lowering of vegetation zones, it likely moved to lower, drier habitats throughout most of the central Rocky Mountains. Adams (1983) reviewed the literature on packrat middens and pollen profiles; the study suggested that life zones descended 300 to 1100 m throughout the southwest and Great Basin from 13,500 to 10,000 ybp. The current separation of *J. scopulorum* and *J. virginiana* appears to have been bridged with the eastward expansion of *J. scopulorum* and the western expansion of *J. virginiana*. Trees of *J. scopulorum* are currently growing in ravines in northeastern New Mexico, while *J. virginiana* has now migrated westward into the Canadian River canyons in the Texas panhandle. The population of *J. scopulorum/virginiana* in Palo Duro Canyon resembles both species and is likely a relictual stand of hybrid origin (Adams, 1983).



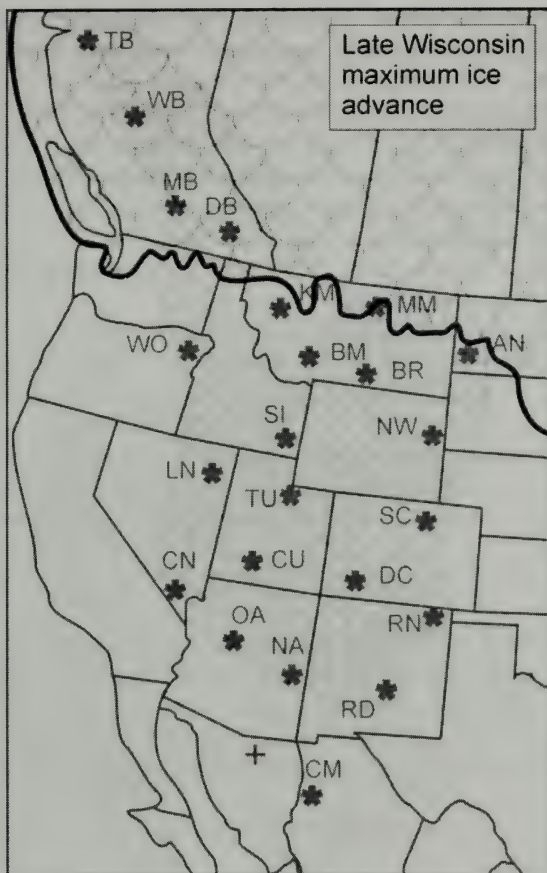


Figure 3. Maximum ice advance during the late Wisconsin (based on Flint, 1971; Crandell, 1971).

With the retreat of the Wisconsin glacial ice, and the subsequent altithermal period 9000 to 5000 ybp (Wells, 1970), *Juniperus* expanded into the drying, higher elevation habitats that it occupies today. Figure 4 shows the proposed post-Pleistocene recolonization of the northern portion of the range of *J. scopulorum*. Based on the terpenoid data, the BC populations could have been recolonized by seed from the Wallowa Mtns. refugium (WA, Fig. 4)

and thence northward to the present day northern-most population at Telkwa, BC (TB). At Telkwa, *J. scopulorum* is found on dry, southeast facing slopes (ca. 45° - 60°).

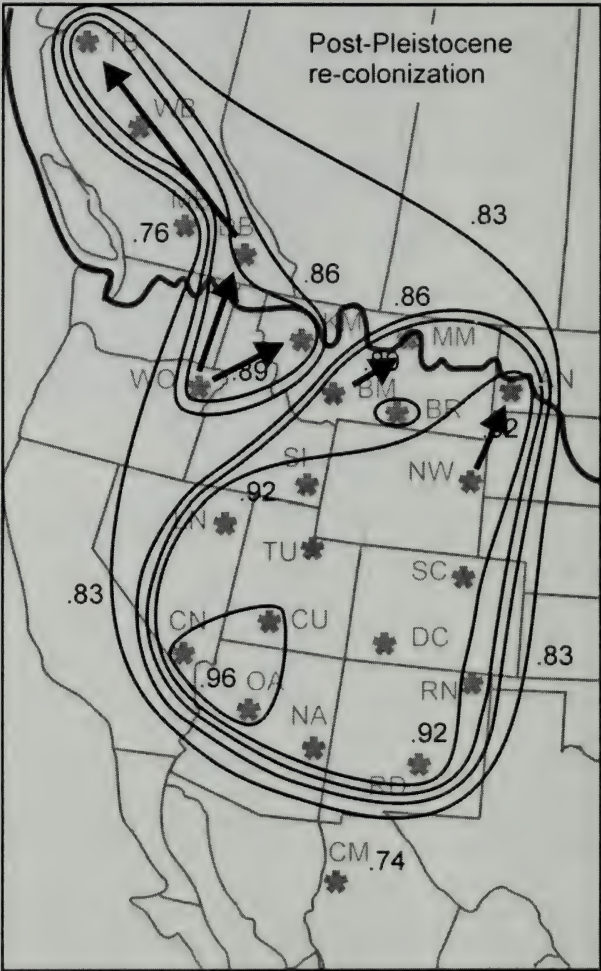


Figure 4. Proposed post-Pleistocene re-colonization of the northern range of *J. scopulorum* during the post-glacial period (9000 - 5000 ybp).



The Kalispell, MT (KM) population shares the divergent terpene nature with the BC and WA populations and is postulated to have arisen by seed from the Wallowa population. Of course, the Wallowa population may have been displaced lower, and perhaps a bit to the south during the Wisconsin. The Amidon, ND (AN) population is typical of the central Rocky Mountains and seems likely to have been derived by seed from the nearest *J. scopulorum* population (perhaps near Newcastle, WY, NW) or any of the scarp land *J. scopulorum* populations to the south.

There is not evidence from the terpene data for the extinction of the central Rocky Mtn. populations; it is likely they persisted near the present day locations at lower, drier elevation sites.

*Juniperus scopulorum* is presently found along running streams in the disjunct populations of northern Mexico. It is possible that the latter have been isolated since the Pliocene (Martin and Harrell, 1957).

### ACKNOWLEDGEMENTS

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**UNDERSTORY VEGETATION OF THORN-SCRUB  
WOODLANDS AT THE CHAPARRAL WILDLIFE  
MANAGEMENT AREA, DIMMIT AND LASALLE COUNTIES,  
TEXAS**

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**ABSTRACT**

The South Texas Plains was originally open savanna dominated by *Prosopis glandulosa* (honey mesquite) along with scattered brushy regions, whereas the ground layer was dominated by short and mid-grasses and forbs. This open savanna has changed to thorn-scrub woodland within the last 150 years, apparently due to anthropogenic forces, including overgrazing and reduced fire frequency. We undertook this study to determine the structure and composition of these thorn-scrub woodlands at the Chaparral Wildlife Management Area (CWMA), Dimmit and LaSalle Counties, Texas, in the northern half of the South Texas Plains. Within this 6,150 ha site the spring and fall ground layer vegetation under various thorn-scrub woodlands and adjacent aerated (roll-chopped) sites were surveyed. Species diversity was relatively high with 318 species of vascular plants encountered at the CWMA. Fern, "fern-allies", and gymnosperms were represented by 4 taxa in 3 families. Of the remaining taxa, 65 were monocots in 8 families, and 249 were dicots in 63 families. Non-native (exotic) species accounted for 17 taxa, about 5% of the species collected. The Poaceae was the most common family with 49 species, Asteraceae was second with 46 species, and the Fabaceae was represented by 32 species. No state endangered or threatened species were encountered. In both the fall and spring

surveys grasses dominated, introduced *Pennisetum ciliaris* (buffelgrass) being very important, followed by *Urochloa ciliatissima* (fringed signalgrass), *Chloris cucullata* (hooded windmill grass), *Bouteloua hirsuta* (hairy grama), *Eragrostis lehmanniana* (Lehmann's lovegrass), *Aristida purpurea* (purple three-awn), and *Digitaria cognata* (fall witchgrass). Forbs were more common during the spring survey with 98 species found in plots. *Phytologia* 93(1): 13-42 (April 1, 2011).

**KEY WORDS:** ground-layer vegetation, importance values, South Texas Plains, species list, spring and fall surveys, thorn-scrub communities.

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The South Texas Plains once supported an open savanna with a ground layer of short and mid-grasses and forbs in which woody vegetation was dominated by *Prosopis glandulosa* (honey mesquite), along with other shrubs and trees. This region also contained a mosaic of rocky, broken uplands that were dominated by relatively dense brushy vegetation. This open savanna has changed to thorn-scrub woodland within the last 150 years, apparently due to anthropogenic forces (Correll and Johnston 1970; Van Auken 2000; Ruthven 2001). Most of these changes involve dramatic increases in native woody taxa that were historically present in low densities (Johnston 1963; Archer et al. 1988; Archer 1989). Along with this change in shrub and tree density there has been a corresponding change in ground layer species. Major shifts in the abundance of herbaceous species were probably the result of increased shading that caused a decrease in prairie species and a corresponding increase in species that were more shade-tolerant, less fire-tolerant, and more tolerant of moisture extremes.

Anthropogenic forces, particularly increased grazing pressure by domestic livestock, fire suppression, and the introduction of exotic species (particularly grasses) have reduced the abundance of native species. These changes resulted in the destruction of the extensive short and mid-grass prairie matrix (Archer et al. 1988; Ruthven et al. 2000; Ruthven 2001). *Prosopis glandulosa* was the pioneer woody species involved in this transition to thorn-scrub woodland, and is currently the common dominant throughout the southwestern United States and adjacent Mexico (Ruthven 2001). Species in two genera of



well-armed legumes (*Senegalia* and *Vachellia*) are also major components of these thorn-scrub woodlands. These two genera are segregates of the genus *Acacia* (*sensu lato*) and are common throughout the arid and semi-arid environments of the South Texas Plains (Isely 1998).

Thorn-scrub woodlands are common at the Chaparral Wildlife Management Area (CWMA). Within this community type the importance and distribution of the associated ground layer species is determined by various biotic and abiotic factors, such as climate, moisture, edaphic conditions, present and past grazing pressures, and fire. The objective of this study was to examine the structure and composition of the ground layer vegetation of the thorn-scrub woodland and adjacent disturbed sites to understand better the importance, distribution, and habitat preferences of these associated ground layer species.

### STUDY AREA

All study sites were at Chaparral Wildlife Management Area (CWMA), Dimmit and LaSalle Counties, Texas (28°20'N, 99°25'W). The CWMA is in the northern half of the South Texas Plains ecological region (Correll and Johnston 1970, Diamond et al. 1987, Ruthven et al. 2000, Ruthven 2001). Located 12 km west of Artesia Wells, the CWMA is deer-proof fenced and about 6,150 ha in size. Purchased in 1969 by the Texas Parks and Wildlife Department, it serves as a research and demonstration area. Domestic livestock have grazed the CWMA since the 18th century (Lehmann 1969). The CWMA utilizes a high intensity, low frequency rotational grazing system with stocking rates of one Animal Unit per 12 ha (Ruthven 2001). Most of the CWMA was chained in 1948 (Ruthven, personal communication). Chaining involves the use of two large tractors with a heavy linked chain connected at each end to each of the tractors. The chain is pulled across the ground, disrupting and pulling out much of the woody vegetation (Lehmann 1984). The land around CWMA is rangeland, most holdings being large cattle ranches.

Hot summers and mild winters characterize the climate of CWMA; short-term droughts are common (Norwine and Bingham 1985). Average daily minimum winter (January) temperature is 5°C,

average daily maximum summer (July) temperature is 37°C, and growing season is 240 to 365 days. Average annual precipitation (1951 to 1978) is 550 mm (Stevens and Arriaga 1985; Cooper et al. 2008). Precipitation patterns are bimodal with peaks in late spring (May and June), and early fall (September and October). Topography is level to gently rolling with an average elevation of 175 m above mean sea level. The thin calcareous soils have low productivity and are dominated by Duval very fine, sandy loams, gently sloping and Duval loamy fine sands, 0 to 5% slope (Gabriel et al. 1994; Stevens and Arriaga 1985). The soil surface layer is reddish brown, slightly acid, very friable, and 0 to 40 cm thick. Also present are shallow limestone ridges (calcareous rises) where soils are mildly to moderately alkaline and have a caliche layer near the surface.

## METHODS

**Floristic Composition:** CWMA was visited five times during the growing seasons of 2001 to 2005. During these visits voucher specimens from all habitat types throughout the CWMA were collected and deposited in the herbaria of the University of Illinois (ILL) and the Illinois Natural History Survey (ILLS), Champaign/Urbana, Illinois. The designation of exotic species follows Correll and Johnston (1970) and Nesom (2008). Nomenclature follows Jones et al. (1997), with the common names mostly from Correll and Johnston (1970).

**Ground Layer Sampling:** In early November of 2002 and again in late May of 2003 transects were located randomly along cardinal compass directions within each community of the four thorn-scrub communities studied. These sites were the same as those used to sample the woody vegetation of thorn-scrub communities that differed in their overstory composition (Seigler et al. 2007), and were originally selected based on the recommendation of CWMA personnel who located sites where the vegetation was mature and least disturbed. All were near level uplands with minimal disturbance, other than grazing. At these four sites (study sites 1A, 2A, 3A, 4A) a single line transect 50 m long was randomly established near the center of the long axis of each community. Adjacent to these four original study sites, CWMA personnel had previously aerated 2 to 4 hectares, with a double/tandem drum aerator pulled by a D7 bulldozer. Presently, aeration is the preferred choice of wildlife managers to improved pastures in south



Texas. The process is similar to roll-chopping, but the blades along the chopper drum are toothed and set at an angle across the face of the large drum rather than a continuous blade running parallel to the face of the drum (Ruthven and Krakauer 2004). This aerated ground had been left undisturbed for about one year. In each of these successional sites (study sites 1B, 2B, 3B, 4B) a single line transect 50 m long was randomly established near the center of the long axis of each community. In all eight sites studied (four aerated and four not aerated) quadrats 1 m<sup>2</sup> in size were located alternately along each transect (n = 50 plots). A random numbers table was used to determine the distance (0 to 9 m) a quadrat was located from the transect line. Species cover was determined using the Daubenmire (1959) cover class system as modified by Bailey and Poulton (1968). The modified Daubenmire cover scale is as follows: class 1 = 0 to 1%; class 2 = >1 to 5%; class 3 = >5 to 25%; class 4 = >25 to 50%; class 5 = >50 to 75%; class 6 = >75 to 95%; class 7 = >95 to 100%. Only ground layer species rooted within the quadrat frame were recorded. Mean cover was determined for each taxon using the mid-point values for each cover class, while Importance Value (IV) was calculated by summing relative cover and relative frequency (total possible 200). Listed below are the eight study sites (Seigler et al. 2007) with the dominant overstory species encountered, these species Importance Values (possible 200), and the GPS coordinates.

Site 1A: *Senegalia greggii* (IV of 68.6), *Opuntia engelmannii* (IV of 28.5), *Vachellia rigidula* (IV of 27.5). 28°20'29"/99°22'47"

Site 1B. Aerated site next to 1A. 28°20'23"/99°22'50"

Site 2A: *Prosopis glandulosa* (IV of 89.0), *Opuntia engelmannii* (IV of 48.8), *Vachellia bravoensis* (IV of 25.9). 28°18'06"/99°21'40"

Site 2B: Aerated site next to 2A. 28°18'11"/99°21'41"

Site 3A: *Opuntia engelmannii* (IV of 56.0); *Prosopis glandulosa* (IV of 52.7); *Vachellia bravoensis* (IV of 27.3). 28°18'07"/99°21'31"

Site 3B: Aerated site next to 3A. 28°18'09"/99°21'28"

Site 4A: *Vachellia rigidula* (IV of 46.8); *Senegalia berlandieri* (IV of 44.3); *Opuntia engelmannii* (IV of 26.7). 28°18'55"/99°20'46"

Site 4B. Aerated site next to 4A. 28°18'57"/99°20'52"

The Sorensen Index of Similarity (ISs) was used to determine the degree of vegetation similarity between the sites surveyed throughout the ICCA (Mueller-Dombois and Ellenberg 1974). This

index utilizes binary data (presence/absence) to measure the similarity in species composition between study sites and is represented by the following equation:  $[ISs = 2C/A+B \times 100]$ , A equals the number of species in the first community, B equals the number of species in the second community, and C equals the number of species common between the two communities. Pairwise comparisons were made between each of the communities examined for both the November 2002 and the May 2003 surveys.

## RESULTS

Species diversity was relatively high with 318 species of vascular plants encountered at CWMA (Appendix I). Fern, “fern-allies”, and gymnosperms were represented by 4 taxa in 3 families. Of the remaining taxa, 65 were monocots in 8 families, and 249 were dicots in 63 families. Non-native (exotic) species accounted for 17 taxa, about 5% of the species collected (Nesom 2008). As is typical of prairie and thorn-scrub vegetation, Poaceae was the most common family with 49 species, Asteraceae was second with 46 species, whereas Fabaceae was represented by 32 species. No state endangered or threatened species were encountered.

**Fall Survey:** Collectively, 72 species were encountered in the plots of the eight sites examined at CWMA during the fall survey (Table 1), based on the highest average importance value of each species (total IV of a species in all study sites). Grasses and grass-like species dominated, introduced *Pennisetum ciliaris* (buffelgrass) being an important component of five study sites, followed by *Urochloa ciliatissima* (fringed signalgrass), *Chloris cucullata* (hooded windmill grass), *Bouteloua hirsuta* (hairy grama), *Eragrostis lehmanniana* (Lehmann’s lovegrass), *Aristida purpurea* (purple three-awn), *Cyperus retroflexus* (flatsedge), and *Digitaria cognata* (fall witchgrass) that were common in four to seven of the sites studied. Common forbs among the top 10 species included *Evolvulus alsinoides* (ojo de víbora) and *Croton glandulosus* (northern croton), with *Tiquilia canescens* (oreja de perro) being common only in Site 4A where it was the most important species with an IV of 83.1 (200 possible). Site 4A was located on a shallow limestone ridge where soils were moderately alkaline and most of the grass species found on the other study sites

were uncommon or absent. This site had a low ISs when compared to the other sites studied in the fall survey (Table 2).

Of the 72 taxa found in the plots examined, 19 taxa were recorded for only one of the eight sites examined (Table 1). Of these species, most were recorded in low number, occurring in only a few plots. Only in Site 4B did two species restricted to only one site have IVs greater than 2.5 [*Tridens muticus* (IV of 19.9) and *Sideroxylon celastrinum* (IV of 3.4)]. An additional 19 taxa were encountered in only two of the study sites. Over half of this group were common components of one study site, and only rarely encountered in another. Only two exotic species were found in the plots: *Eragrostis lehmanniana* and *Pennisetum ciliaris* (Table 1). Both species had relatively high IVs and are commonly planted for forage (Ruthven 2001, Lonard and Judd 2002).

**Spring Survey:** Ninety-eight species (excluding the grass taxa that are listed as a species group) were encountered in the plots of the eight sites examined at the CWMA during the spring survey (Table 3). Based on the importance value of each species (total IV of a species on all study sites) members of grass-like species dominated. As grasses were mostly dormant or vegetative, and the same species that we had encountered in the fall survey, we treated the grass taxa as a species group. Together these species usually accounted for more than one-quarter of the total IV in each of the study sites. Dominant forbs in most study sites were *Coreopsis nuecensis* (tick-seed), *Gamochaeta purpurea* (purple cudweed), *Aphanostephus riddellii* (Riddell's lazy daisy), *Oxalis dillenii* (yellow wood sorrel), *Plantago hookeriana* (tallow weed), and *Nothoscordum bivalve* (crow-poison), whereas *Oenothera grandis* (showy ragged evening primrose) and *Tiquilia canescens* (oreja de perro) were common only on the limestone ridge of Site 4A. As in the fall survey, Site 4A had a low ISs when compared to the other sites studied (Table 4).

Of the 98 taxa found in the plots examined, 32 were recorded for only one of the eight sites examined (Table 3). Of these species, most were recorded in low numbers, occurring in only a few plots. Only in Sites 4A and 4B was a species restricted to only one site with an IV greater than 2.5 [*Houstonia croftiae* (IV of 4.4) and *Draba*



*cuneifolia* (IV of 3.9)]. An additional 14 taxa were encountered in only two of the study sites. Over half of this group were common components of one study site, and only rarely encountered in one other. Except for the two grasses reported in the fall survey no other exotic species were encountered in the plots.

## DISCUSSION

The thorn-scrub vegetation of CWMA and surrounding area is representative of that associated with the South Texas Plains (South Texas Brush Country or Tamaulipan Brushlands) (Johnston 1963, Correll and Johnston 1970). Throughout most of this rangeland, *Prosopis glandulosa* is the dominant woody species, with about 10 to 15 other woody or large succulent, mostly thorny species, varying in abundance and composition. At CWMA, *P. glandulosa* is usually the dominant woody species, but, on some areas, other woody legumes are dominant or co-dominant, particularly various species of *Senegalia* and *Vachellia* (*Acacia s.l.*). This woodland community, where dominant trees are more than 3 m tall and formed a 26-60 percent canopy, would be equivalent to the Deciduous Woodland, Mesquite-Huisache Series (*Prosopis glandulosa-Vachellia farnesiana*) of Diamond et al. (1987) with other thorny legume species replacing *V. farnesiana*.

The ground layer vegetation at the study sites at CWMA was mostly similar, many of the species encountered being found on most of the eight study sites. In particular, the associated aerated communities for each of the four thorn-scrub communities studied consistently had ISs between 56.6 and 74.1 for the fall survey (Tables 2), and 65.5 and 83.5 for the spring survey (Table 4). These aerated communities were cleared two years previous to the study but many of the ground layer species were present, many with similar IVs. The few exotic species present (*Pennisetum ciliaris*, *Eragrostis lehmanniana*) mostly did not show much of an increase in frequency, cover, or IV on most aerated communities. In contrast, *Pennisetum ciliaris*, the dominant exotic grass found on some of the study sites did increase in IV from 50.6 to 90.6 on Site 3 (Table 1). Site 3 was slightly drier with *Opuntia engelmannii* the dominant overstory species, which may have accounted for this increase due to lower grazing pressure.

On all study sites, the soil texture was relatively uniform, being sandy loams with 61 to 75% sand, 12 to 20% silt, and 11 to 19% clay, and none were saline. Soils of Sites 1, 2, and 3 were mildly to strongly acidic whereas soils at Site 4, in contrast, were from a calcareous ridge and were mildly to moderately alkaline. Although all sites had relatively high levels of available calcium, site 4, was significantly higher ( $P < 0.0001$ ) (Seigler et al. 2007). These differences in soil pH were probably responsible for some differences recorded in ground layer species distribution at CWMA.

The *Senegalia greggii*/*Opuntia engelmannii* community had a restricted distribution at CWMA (Site 1) being common on dry sandy ridges. At this location *S. greggii* was the dominant member of the community, accounting for one-third of the total IV. This community, which is probably maintained by fire, grazing, and sandy soil, is classified as the Catclaw Acacia Series, Deciduous Scrubland (*Senegalia greggii*). The woody vegetation at this site was short with only a few individuals being more than 2 m tall while the canopy cover was estimated at 25 to 30 percent (Seigler et al. 2007). Both Site 2 and 3 are similar to the Catclaw Acacia Series, being classified as the Deciduous Woodland, Mesquite-Huisache Series (*Prosopis glandulosa*-*Vachellia farnesiana*) of Diamond et al. (1987) with *Opuntia engelmannii* being common and *Vachellia bravoensis* replacing *V. farnesiana*. The ground layer vegetation in both the spring and fall surveys of the Catclaw Acacia Series (Site 1) and the Mesquite-Huisache Series (Sites 2 and 3) were similar in both the spring and fall surveys with the ISs always 48 or above (Tables 2 and 4).

*Vachellia rigidula* and *Senegalia berlandieri* dominated limestone ridges (calcareous rises) at CWMA (Site 4). This community was dominated by shrubs or small trees 0.5 to 3 m tall that formed 26 percent or more of the total canopy and was equivalent to the Deciduous Shrubland, Blackbrush Series (*Vachellia rigidula*) of Diamond et al. (1987). Though many of the ground layer species encountered were associated with all of the study sites, some of the species associated with the limestone ridges were found only associated with Sites 4A and B, or were much more common on those sites. Ground layer species mostly associated with these limestone ridges included *Aristida purpurea*, *Astragalus nuttalianus*, *Bouteloua trifida*,

*Dyssodia pentachaeta*, *Justicia pilosella*, *Lepidium lasiocarpum*, *Menodora heterophylla*, *Nama jamaicense*, *Nothoscorum bivalve*, *Oenothera grandis*, *Spermolepis echinata*, *Tiquilia canescens*, and *Tridens muticus* (Table 1 and 3). Of these, *Aristida purpurea* and *Tridens muticus* were only found at aerated Site 4B of the limestone ridge. The ISs for Site 4A during the fall survey was 17.0 to 30.8, less than half of the ISs recorded for the other sites (Table 2).

In a study on the CWMA involving species distribution under *Prosopis glandulosa* many of the common species found were the same we reported as common in the present study. Ruthven's (2001) list included *Bouteloua hirsuta*, *Chloris cucullata*, *Digitaria cognata*, *Eragrostis lehmanniana*, *Eragrostis secundiflora*, *Panicum capillarioides*, *Paspalum setaceum*, *Urochloa ciliatissima*, and *Evolvulus alsinoides* (Table 1). In a later study involving the species abundance and distribution after aeration, Ruthven and Krakauer (2004) found that aeration maintained woody species diversity, that the woody cover increased very rapidly after aeration, and that grass and forb richness, diversity, and evenness did not differ significantly among treatments.

The reasons for the continued prevalence of thorn-scrub woodland communities along with their associated ground-layer species are not entirely clear, but overgrazing and fire suppression were probably the primary causes (Van Auken 2000). At the time of European settlement much of the South Texas Plains was covered with open savanna and a dense groundcover of grasses and forbs. Many of the herbaceous species of this savanna were associated with the short and mid-grass prairie of central Texas. At that time wildfires were undoubtedly frequent and of sufficient intensity to prevent or delay encroachment by native woody species. However, overgrazing by livestock reduced the fuel load. This associated with fire suppression allowed for a significant decrease in fire frequency creating ideal conditions for the rapid explosion of native invaders. With the development of thorn-scrub communities, the resulting canopy closure, an increased water loss due to more rapid run-off resulted in a decrease of the integrity of the prairie. This decrease in the prairie community structure resulted in a corresponding loss in biodiversity.



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Table 1. Importance value (IV) of the ground layer species encountered in a fall survey (2002) of thorn-scrub vegetation (1A, 2A, 3A, 4A) and adjacent aerated sites (1B, 2B, 3B, 4B) at the Chaparral Wildlife Management Area, Dimmit and LaSalle Counties, Texas. Also listed is the cover of bare ground and litter, and the total number of species in the plots of each site. Only species with an IV of  $\pm 4.5$  for a site are included. \* non-native species.

Species / Sites	1A	1B	2A	2B	3A	3B	4A	4B
* <i>Pennisetum ciliaris</i>	--	--	25.6	33.9	50.6	90.6	--	23.2
<i>Urochloa ciliatissima</i>	18.7	23.6	33.6	30.6	28.9	10.8	--	--
<i>Evolvulus alsinoides</i>	20.3	19.7	20.1	23.1	21.8	15.2	1.7	2.1
<i>Croton glandulosus</i>	12.9	12.2	29.9	44.9	10.1	2.6	--	--
<i>Chloris cucullata</i>	0.9	6.6	22.3	14.0	9.4	21.5	--	12.4
<i>Tiquilia canescens</i>	--	--	--	--	--	--	83.1	3.0
<i>Bouteloua hirsuta</i>	38.3	24.5	7.8	10.2	--	--	--	--
* <i>Eragrostis lehmanniana</i>	20.6	30.4	2.5	1.2	10.3	2.2	4.4	1.1
<i>Aristida purpurea</i>	5.1	3.8	4.8	--	6.1	1.0	--	46.4
<i>Cyperus retroflexus</i>	6.7	7.1	7.2	8.1	8.0	1.3	--	7.7
<i>Digitaria cognata</i>	2.2	10.4	2.9	4.6	4.0	1.8	--	17.4

Table 1 (continued).

Species / Sites	1A	1B	2A	2B	3A	3B	4A	4B
<i>Palafoxia texana</i>	5.1	24.7	2.1	1.6	3.0	0.6	--	0.7
<i>Bouteloua trifida</i>	--	--	--	--	--	--	20.7	7.0
<i>Diodia teres</i>	29.8	2.4	--	--	--	--	--	--
<i>Dyssodia pentachaeta</i>	--	0.8	--	--	--	--	23.8	2.5
<i>Nothoscordum bivalve</i>	--	--	--	--	3.2	--	20.8	--
<i>Sida abutifolia</i>	--	0.9	2.6	2.2	3.4	6.0	5.3	1.8
<i>Paspalum setaceum</i>	1.4	--	6.8	--	7.5	0.6	--	3.8
<i>Tridens muticus</i>	--	--	--	--	--	--	--	19.9
<i>Eragrostis secundiflora</i>	3.3	7.4	0.5	--	3.6	--	2.1	1.8
<i>Setaria texana</i>	--	--	--	3.7	--	--	2.1	11.0
<i>Panicum capillarioides</i>	--	--	2.6	--	1.1	10.7	1.7	--
<i>Setaria reverchonii</i>	0.9	8.4	3.9	--	1.7	0.7	--	--
<i>Cenchrus spinifex</i>	7.1	0.8	2.1	4.5	0.6	--	--	--
<i>Opuntia engelmannii</i>	--	1.0	5.5	--	5.4	0.7	--	2.5
<i>Mollugo verticillata</i>	0.9	--	3.0	1.7	4.5	3.5	--	0.6
<i>Justicia pilosella</i>	--	--	--	--	--	1.0	8.0	2.3
<i>Phyllanthus polygonoides</i>	0.9	--	--	--	--	10.1	--	--
<i>Eragrostis curtipedicillata</i>	--	--	1.0	--	1.1	2.2	--	6.6
<i>Eragrostis sessilispica</i>	5.3	--	1.6	3.8	--	--	--	--
<i>Menodora heterophylla</i>	--	--	--	--	--	--	9.5	1.1
<i>Chamaecrista fasciculata</i>	2.3	8.0	--	--	--	--	--	--
<i>Dalea nana</i>	6.3	0.8	--	--	--	--	--	--



Table 1 (continued).

Species / Sites	1A	1B	2A	2B	3A	3B	4A	4B
<i>Ambrosia</i>	--	--	--	--	0.6	6.1	--	--
<i>psilostachya</i>								
Other species	11.0	6.5	11.6	11.9	15.1	10.8	16.8	25.1
Total	200	200	200	200	200	200	200	200
Bare ground/litter cover	51.80	44.78	47.80	32.53	49.77	28.09	79.21	39.63
Total species for each site	27	27	30	23	32	27	20	36

Table 2. Sorensen Index of Similarity of the ground layer vegetation at the eight communities examined in a fall survey (2002) at the Chaparral Wildlife Management Area, Dimmitt and LaSalle Counties, Texas.

Site	1A	1B	2A	2B	3A	3B	4A
1A							
1B	74.1						
2A	63.2	56.1					
2B	52.0	48.0	56.6				
3A	61.0	57.6	77.4	58.2			
3B	48.1	48.1	63.2	48.0	64.4		
4A	17.0	25.5	20.0	27.9	30.8	29.8	
4B	34.9	38.1	45.5	47.5	47.1	50.8	57.1

Table 3. Importance value (IV) of the ground layer species encountered in a spring survey (2003) of thorn-scrub vegetation (1A, 2A, 3A, 4A) and adjacent aerated sites (1B, 2B, 3B, 4B) at the Chaparral Wildlife Management Area, Dimmit and LaSalle Counties, Texas. Also listed is the cover of bare ground and litter, and the total number of species in plots of each site. Only species with an IV of  $\pm 4.5$  for a site are included. \* non-native species

Species	1A	1B	2A	2B	3A	3B	4A	4B
Total grasses (living/dead)	67.6	52.6	59.6	45.2	56.7	74.2	39.0	77.7
<i>Coreopsis nucensis</i>	34.4	49.2	6.1	35.1	22.3	0.6	--	--
<i>Gamochaeta purpurea</i>	--	--	13.2	15.2	8.0	23.6	--	4.3
<i>Aphanostephus riddellii</i>	5.7	8.2	5.6	8.9	6.2	0.5	16.5	9.8
<i>Oxalis dillenii</i>	0.6	2.9	13.1	8.0	9.0	13.9	1.1	10.8
<i>Plantago hookeriana</i>	9.6	7.7	11.5	8.1	13.1	1.8	--	1.1
<i>Oenothera grandis</i>	--	--	0.2	1.1	1.0	0.3	26.9	11.8
<i>Nothoscordum bivalve</i>	6.7	5.5	3.8	1.9	4.8	0.2	7.1	0.2
<i>Nuttallanthus tenanus</i>	--	1.5	4.7	9.2	5.6	7.9	--	0.4
<i>Evolvulus alsinoides</i>	6.8	5.5	3.1	5.6	4.9	2.1	--	0.2
<i>Dyssodia tenuiloba</i>	0.6	0.3	3.0	0.3	8.4	5.7	3.3	6.5
<i>Cyperus retroflexus</i>	6.0	4.6	5.4	2.3	5.6	2.2	--	0.2
<i>Tiquilia canescens</i>	--	--	--	--	--	--	25.7	0.6
<i>Evax prolifera</i>	3.3	1.9	5.1	0.9	1.0	0.9	6.8	5.3
<i>Lesquerella argyraea</i>	6.7	6.3	4.7	4.5	1.9	0.2	--	--
<i>Ambrosia confertiflora</i>	--	--	6.7	--	4.3	11.1	--	--
<i>Chamaesaracha coniodes</i>	6.7	9.9	--	--	--	--	4.7	0.8
<i>Palafoxia texana</i>	1.3	9.2	1.9	1.4	6.3	1.1	0.1	0.4
<i>Thelesperma filifolium</i>	8.7	7.3	2.2	1.2	0.3	0.2	--	--

Table 3 (continued).

<i>Gaura mckelveyae</i>	0.6	--	4.3	2.3	3.5	5.8	0.3	1.3
<i>Lepidium virginicum</i>	--	--	4.4	6.3	2.6	2.8	--	1.5
<i>Triodanis perfoliata</i>	--	0.5	7.2	4.8	3.2	1.4	--	--
<i>Dalea nana</i>	8.7	4.4	2.4	--	--	0.2	1.3	--
<i>Sida abutilifolia</i>	1.2	1.2	1.7	2.6	1.9	1.9	4.7	1.3
<i>Plantago virginica</i>	--	--	3.5	0.5	3.1	0.8	6.7	1.1
<i>Parietaria pensylvanica</i>	--	--	1.0	2.9	0.3	7.4	--	4.0
<i>Nama jamaicense</i>	--	--	--	0.5	--	--	2.3	12.4
<i>Descurainia pinnata</i>	--	--	--	3.2	--	8.5	--	1.8
<i>Astragalus nuttalianus</i>	0.2	1.1	1.2	0.9	2.5	--	4.6	2.0
<i>Lepidium lasiocarpum</i>	0.2	--	--	--	--	--	5.9	5.9
<i>Talinum parviflorum</i>	4.7	1.4	2.4	0.3	2.1	0.7	--	--
<i>Spermolepis echinata</i>	--	--	--	--	--	--	7.1	4.1
<i>Euphorbia micromera</i>	1.0	0.2	1.3	0.2	1.3	--	5.8	1.1
<i>Linum imbricatum</i>	3.9	4.9	1.3	0.2	0.6	--	--	--
<i>Gymnosperma glutinosum</i>	--	--	--	--	--	--	0.2	9.5
<i>Menodora heterophylla</i>	--	--	--	--	--	--	5.8	1.0
Other species	14.8	13.7	19.4	26.4	19.5	24.0	24.1	22.9
Total	200	200	200	200	200	200	200	200
Bare ground/litter	33.1	17.0	21.2	8.5	25.4	6.8	29.3	12.5
Total species for each site	35	39	44	47	42	43	39	53

Table 4. Sorensen Index of Similarity of the ground layer vegetation at eight communities examined in a spring survey (2003) at the Chaparral Wildlife Management Area, Dimmitt and LaSalle Counties, Texas.

Site	1A	1B	2A	2B	3A	4B	4A
1A							
1B	75.7						
2A	58.2	65.1					
2B	48.8	55.8	83.5				
3A	54.5	61.7	88.4	83.1			
3B	46.2	53.7	75.9	77.8	75.3		
4A	40.5	43.6	43.4	41.9	44.4	39.0	
4B	38.6	39.1	51.5	62.0	54.7	54.2	65.2

Appendix I. Vascular plant species at Chaparral Wildlife Management Area, Dimmitt and LaSalle Counties, listed alphabetically by family under major plant groups. Collecting numbers after each name are those of D. S. Seigler, and deposited in the herbarium of the University of Illinois (ILL). Specimens by L.R.Phillippe (P before the number) are deposited in the Illinois Natural History Survey herbarium (ILLS). Nomenclature follows Jones et al. (1997). (\*exotic species)

## FERN AND FERN-ALLIES

### MARSILEACEAE

*Marsilea vestita* Hooker & Greville; 15043, 15490

### PTERIDACEAE

*Astrolepis cochisensis* (Goodding) Benham & Windham; 15642

*Cheilanthes alabamensis* (Buckley) Kunze; 15643



**GYMNOSPERMS**

**EPHEDRACEAE**

*Ephedra antisiphilitica* C.A. Meyer; 14911, 15505

**MONOCOTS**

**AGAVACEAE**

*Agave americana* L.; 15639

*Yucca constricta* Buckley; 15154

*Yucca treculeana* Carrière; 15463, 15683

**COMMELINACEAE**

*Commelina erecta* L.; 14868, 15601

**CYPERACEAE**

*Carex tetrastachya* Scheele; 15200, 15607

*Cyperus acuminatus* Torrey & Hooker; 15046

*Cyperus echinatus* (L.) A. Wood; 15045

*Cyperus retroflexus* Buckley; 15005, 15377

*Eleocharis palustris* (L.) Römer & Schultes; 15497

*Fimbristylis vahlii* (Lamark) Link; 15126

*Schoenoplectus saximontanus* (Fernald) Raynal; 15488

**LILIACEAE**

*Cooperia drummondii* Herb; 15103

*Nothoscordum bivalve* (L.) Britton; 15184

**NAJADACEAE**

*Najas guadalupensis* (Sprengel) Magnus; 15625

**POACEAE**

*Agrostis hyemalis* (Walter) B.S.P.; 15487

*Aristida purpurea* Nuttall var. *purpurea*; 15216, 15397

*Aristida purpurea* Nuttall var. *wrightii* (Nash) Allred; 14935

*Bothriochloa barbinodis* (Lagasca) Herter; 14937, 15349

*Bouteloua barbata* Lagasca; 15085

*Bouteloua hirsuta* Lagasca; 15108, 15415

*Bouteloua trifida* Thurber; 15388, 15519

\**Bromus catharticus* Vahl; 15174

*Cenchrus spinifex* Cavanilles; 14944

- Chloris cucullata* Bischoff; 14918, 15083  
\**Cynodon dactylon* (L.) Persoon; 14963  
\**Dactyloctenium aegyptium* (L.) Beauvois; 15086  
\**Dichanthium annulatum* (Forsskål) Stapf; 15034  
*Digitaria californica* (Bentham) Henrard; 14936  
*Digitaria ciliaris* (Retzius) Köler; 15074, 15208  
*Digitaria cognata* (Schultes) Pilger; 15206, 15373  
*Eragrostis curtipedicellata* Buckley; 14989, 15427  
\**Eragrostis lehmanniana* Nees; 15146, 15413  
*Eragrostis pectinacea* (Michaux) Nees var. *miserrima* (Fournier) J. Reeder; 15572  
*Eragrostis reptans* (Michaux) Nees; 15127  
*Eragrostis secundiflora* Presl; 15082, 15414  
*Eragrostis sessilispica* Buckley; 14971  
*Heteropogon contortus* (L.) Beauvois; 14987, 15365  
*Leptochloa dubia* (Kunth) Nees; 15366  
*Nasselia leucotricha* (Trinius & Ruprecht) Pohl; 15655  
\**Panicum antidotale* Retzius; 15348  
*Panicum capillarioides* Vasey; 15368, 15376  
*Panicum hallii* Vasey var. *filipes* (Scribner) F. Waller; 15396, 15481  
*Panicum hians* Elliott; 15048  
*Panicum nodatum* Hitchcock & Chase; 15198  
*Panicum oligosanthos* Schultes; 15375  
*Panicum virgatum* L.; 15736  
*Pappophorum bicolor* Fournier; 15390  
*Pappophorum vaginatum* Buckley; 15205  
*Paspalum lividum* Trinius; 15745  
*Paspalum setaceum* Michaux; 14947, 15197  
\**Pennisetum ciliaris* (L.) Link; 14919, 15351  
*Setaria firmula* (Hitchcock & Chase) Pilger; 15426  
*Setaria leucopila* (Scribner & Merrill) K. Schumann; 15196, 15733  
*Setaria macrostachya* Kunth; 15132, 15733  
*Setaria pumila* (Poiret) Römer & Schultes; 15047  
*Setaria reverchonii* (Vasey) Pilger; 14970  
*Setaria texana* W. Emery; 15395  
\**Sorghum halapense* (L.) Persoon; 15011  
*Sporobolus cryptandrus* (Torrey) A. Gray; 15369  
*Trichloris pluriflora* Fournier; 14934, 15670  
*Tridens eragrostoides* (Vasey & Scribner) Nash; 15199, 15389

*Tridens muticus* (Torrey) Nash; 14976, 15403  
*Urochloa ciliatissima* (Buckley) Webster; 15360, 15416

### **PONTEDERIACEAE**

*Heteranthera limosa* (Swartz) Willdenow; 15747

### **POTAMOGETONACEAE**

*Potamogeton nodosus* Poirét; 15489

### **DICOTS**

#### **ACANTHACEAE**

*Carlowrightia texana* Henrickson & Daniel; 15423  
*Justicia pilosella* (Nees) Hilsenbeck; 14958, 15387  
*Ruellia nudiflora* (A. Gray) Urban var. *runyonii* (Tharp & Barkley) B. L. Turner; 15201

#### **AMARANTHACEAE**

*Alternanthera caracasana* Kunth; 15750  
*Amaranthus albus* L.; 15001, 15120  
*Froelichia floridana* (Nuttall) Moquin; 14865, 14959  
*Froelichia gracilis* (Hooker) Moquin; 15101, 15678  
*Gossypianthes lanuginosus* (Poirét) Moquin; 15562  
*Tidestromia lanuginosa* (Nuttall) Standley; 15121, 15372

#### **ANACARDIACEAE**

*Rhus microphylla* Engelman; 15677

#### **APIACEAE**

*Bowlesia incana* Ruiz & Pavón; 15483  
*Daucus pusillus* Michaux; 15159, 15604  
*Spermolepis echinata* (DC.) Heller; 15167, 15615

#### **ARISTOLOCHIACEAE**

*Aristolochia erecta* L.; 15177, 15580, 15595

#### **ASCLEPIADACEAE**

*Asclepias emoryi* (Greene) Vail; 14962  
*Cynanchum barbigerum* (Scheele) Shinnars; 14902, 15420  
*Cynanchum laeve* (Michaux) Persoon; 15744

*Cynanchum racemosum* (Jacquin) Jacquin var. *unifarium* (Scheele) Sundall; 15128

*Matelea gonocarpus* (Walter) Shinnars; 15425

*Matelea parviflora* (Torrey) Woodson; 15004

## ASTERACEAE

*Acourtia runcinata* (D. Don) B. L. Turner; 15641

*Amblyolepis setigera* DC.; 15568, 15623

*Ambrosia confertiflora* DC.; 14923, 15593

*Ambrosia psilostachya* DC.; 14977, 15092

*Aphanostephus riddellii* Torrey & Gray; 15540

*Aphanostephus ramosissimus* DC.; 15362

*Baccharis neglecta* Britton; 15624, 15734

*Berlandiera texana* DC.; 15651

\**Calyptocarpus vialis* Lessing; 15089

*Centaurea americana* Nuttall; 15661

*Chloracantha spinosa* (Benthams) Neson; 15050

*Cirsium texanum* Buckley; 14972, 15496

*Conyza canadensis* (L.) Cronq. var. *glabrata* (Gray) Cronquist; 15006

*Coreopsis nuecensis* Heller; 15538

*Coreopsis tinctoria* Nuttall; 14893, 15565

*Dichaetophosa campestris* A. Gray; 15158, 15898

*Dyssodia pentachaeta* (DC.) Robinson; 15166, 15393

*Dyssodia tenuiloba* (DC.) Robinson; 14877, 15567

*Evax prolifera* DC.; 15178

*Florestina tripteris* DC.; 14898, 15370

*Gaillardia pulchella* Fougereux; 14892, 15590

*Gamochaeta purpurea* (L.) Cabrera; 15051

*Gutierrezia texana* (DC.) Torr. & Gray var. *glutinosa* (Schauer) Lane; 14909

*Gymnosperma glutinosum* (Sprengel) Lessing; 14973

*Helenium linifolium* Rydberg; 14884

*Helianthus annuus* L.; 15743

*Helianthus debilis* Nuttall; 14864, 15742

*Heterotheca subaxillaris* (Lamarck) Britton & Rusby; 15075

*Hymenopappus scabiosaeus* L'Heritier var. *corymbosus* (Torrey & Gray) B. L. Turner; 15645

*Krigia occidentalis* Nuttall; 15637

*Liatis mucronata* DC.; 14993, 15131



*Melampodium cinereum* DC.; 14879, 15535  
*Palafoxia texana* DC.; 14897, 15170  
*Parthenium confertum* A.Gray; 15384, 15516  
*Pseudognaphalium obtusifolium* (L.) Hilliard & Burt; 15647  
*Pyrrhopappus carolinianus* (Walter) DC.; 15500  
*Pyrrhopappus pauciflorus* (D. Don) DC.; 14956, 15611  
*Ratibida columnifera* (Nuttall) Wooton & Standley; 15009  
*Senecio ampullaceus* Hooker; 15586  
*Simsia calva* (Engelmann & Gray) A.Gray; 15023  
*\*Sonchus aspera* (L.) Hill; 15168  
*Thelesperma burridgeanum* (Regel, Körnicke & Rach) Blake; 14891  
*Thelesperma filifolium* (W. Hooker) A.Gray; 15646a  
*Verbesina encelioides* (Cavanilles) A.Gray; 14863  
*Verbesina microptera* DC.; 15385  
*Xanthisma texanum* DC.; 15371

## BORAGINACEAE

*Cordia boissieri* A.DC.; 15095  
*Cryptantha texana* (A. DC.) Greene; P35386  
*Heliotropium procumbens* Miller; 15125, 15664  
*Heliotropium texanum* I.M. Johnston; 15209, 15355, 15411  
*Lappula occidentalis* (Watson) Greene; 15480, 15650  
*Lithospermum incisum* Lehmann; 15189  
*Tiquilia canescens* (DC.) Richardson; 14933

## BRASSICACEAE

*Arabis petiolaris* (A.Gray) A.Gray; 15465  
*Descurainia pinnata* (Walter) Britton; 15162, 15513  
*\*Diploaxis muralis* (L.) DC.; 15872  
*Draba cuneifolia* Torrey & Gray; 15632, 15870  
*Lepidium lasiocarpum* Torrey & Gray; 15190  
*Lepidium virginicum* L.; 14926  
*Lesquerella argyraea* (A.Gray) Watson; 14881, 15539  
*Lesquerella lasiocarpa* (A.Gray) Watson; 15485, 15618  
*Rorippa teres* (Michaux) Stuckey; 15493  
*\*Sisymbrium irio* L.; 15175, P36371

## BUDDLEJACEAE

*Polypremum procumbens* L.; 15740

**CACTACEAE**

*Ancistocactus scheeri* (Salm-Dyck) Britt. & Rose; 15156

*Echinocereus enneacanthus* Engelm.; 15119

*Opuntia engelmannii* Salm-Dyck; 15012

*Opuntia leptocaulis* DC.; 15024

**CALLITRICHACEAE**

*Callitriche terrestris* Rafinesque; 15869

**CAMPANULACEAE**

*Triodanis perfoliata* (L.) Niewland; 15550, 15648

**CAPPARACEAE**

*Koeberlinia spinosa* Zuccarini; 15113

*Polanisia dodecandra* (L.) DC. subsp. *riograndensis* Iltis; 14946

**CARYOPHYLLACEAE**

*Loeflingia squarrosa* Nuttall; 15557

*Silene antirrhina* L.; 15173, 15596

\**Stellaria media* (L.) Villars; 15650

**CELASTRACEAE**

*Schaefferia cuneifolia* A.Gray; 14912, 15510

**CHENOPODIACEAE**

*Chenopodium berlandieri* Moquin; 14928, 15666

**CONVOLVULACEAE**

*Convolvulus equitans* Benth; 14996, 15574

*Evolvulus alsinoides* (L.) L.; 14984, 15381

*Evolvulus sericeus* Swartz; 15556 15638

*Ipomoea cordatotriloba* Dennstaedt; 14955, 15424

**CUCURBITACEAE**

*Ibervillea lindheimeri* (A.Gray) Greene; 14983

*Ibervillea tenuisecta* (A.Gray) Small; 15118

**CUSCUTACEAE**

*Cuscuta gronovii* Willdenow; 14931, 14978

**EBENACEAE**

*Diospyros texana* Scheele; 14874, 15553

**EUPHORBIACEAE**

*Argythamnia neomexicana* Müller of Aargau; 15394, 15617

*Bernardia myricifolia* (Scheele) Watson; 15755, P36474

*Croton capitatus* Michaux; 14995, 15123

*Croton glandulosus* L.; 15042, 15357

*Croton lindheimerianus* Scheele; 14896

*Euphorbia micromera* P. Boissier; 14924, 15522

*Jatropha dioica* Cervantes; 14951, 15016

*Phyllanthus polygonoides* Sprengel; 14975

**FABACEAE**

*Acaciella angustissima* (Miller) Britton & Rose; 15007, 15741

*Aeschynomene indica* L.; 15746

*Astragalus nuttallianus* DC. var. *austrinus* (Small) Barneby; 15476

*Astragalus nuttallianus* DC. var. *nuttallianus*; 15619

*Chamaecrista fasciculata* (Michaux) Greene; 14895, 15093

*Dalea emarginata* (Torrey & Gray) Shinnery; 14880, 15542

*Dalea nana* Torrey; 14980, 15406

*Dalea pogonathera* A.Gray; 14979, 15537

*Desmanthus virgatus* (L.) Willdenow; 15033, 15739

*Eysenhardtia texana* Scheele; 14953

*Indigofera miniata* Ortega; 15096

*Lupinus texensis* Hooker; 15180, 15585

\**Medicago polymorpha* L.; 15171, 15649

\**Melilotus indicus* (L.) Allioni; 15646b

*Mimosa latidens* (Small) B.L. Turner; 14887, 15183

*Neptunia pubescens* Benthams; 15036

*Parkinsonia aculeata* L.; 15044

*Parkinsonia texana* (A.Gray) Watson; 14907

*Prosopis glandulosa* Torrey; 14929, 15134

*Senegalia berlandieri* (Benthams) Britton & Rose; 14905

*Senegalia x emoryana* (Benthams) Britton & Rose; 15401

*Senegalia greggii* (A.Gray) Britton & Rose; 14890

*Senegalia roemeriana* (Scheele) Britton & Rose; 14954

*Senna lindheimeriana* (Scheele) Irwin & Barneby; 14949

*Senna roemeriana* (Scheele) Irwin & Barneby; 15620

- Tephrosia lindheimeri* A.Gray; 14894  
*Vachellia bravoensis* (Isley) Seigler & Ebinger; 14889  
*Vachellia farnesiana* (L.) Wight & Arnott; 14967  
*Vachellia rigidula* (Bentham) Seigler & Ebinger; 14994  
*Vachellia rigidula x schaffneri*; 15114  
*Vicia ludoviciana* Nuttall; 15470, 15635  
*Zornia bracteata* J. F. Gmelin; 14878, 15579

## **FAGACEAE**

- Quercus virginiana* Miller; 15097

## **FUMARIACEAE**

- Corydalis aurea* Willdenow var. *aurea*; 15554

## **GENTIANACEAE**

- Sabatia campestris* Nuttall; 14986

## **GERANIACEAE**

- Erodium texanum* A. Gray; 15520

## **HYDROPHYLLACEAE**

- Nama hispidum* A.Gray; 14886, 15605  
*Nama jamaicense* L.; 15495, 15633  
*Nama stenocarpum* A.Gray; 15663, P35259  
*Phacelia congesta* Hooker; 14966, 15501

## **KRAMERIACEAE**

- Krameria lanceolata* Torrey; 14871, 15561

## **LAMIACEAE**

- Monarda punctata* L.; 14888  
*Salvia ballotiflora* Bentham; 15018, 15582  
*Scutellaria drummondii* Bentham var. *drummondii*; 15502  
*Stachys crenata* Rafinesque; 15494, 15662

## **LINACEAE**

- Linum berlandieri* Hooker; 14908, 15546  
*Linum imbricatum* (Rafinesque) Shinnars; 14872, P35341



## **MALVACEAE**

- Abutilon fruticosum* Guillemain & Perrottet; 15399  
*Abutilon wrightii* A.Gray; 15606  
*Herissantia crispa* (L.) Brizicky; 15419, 15428  
*Malvastrum coromandelianum* (L.) Garcke; 15203  
*Rhynchosida physocalyx* (A.Gray) Fryxell; 15603, 15610  
*Sida abutifolia* Miller; 14938, 15398  
*Sida ciliaris* L.; 15738a  
*Sida lindheimeri* Engelman & A.Gray; 15410  
*Sida tragiifolia* A.Gray; 15211, 15674  
*Sidastrum paniculatum* (L.) Fryxell; 15409  
*Sphaeralcea pedatifida* (A.Gray) A.Gray; 14915, 15511

## **MOLLUGINACEAE**

- Glinus radiatus* (Ruiz & Pavón) Rohrbach; 15130  
*Mollugo verticillata* L.; 14997, 15087

## **NYCTAGINACEAE**

- Acleisanthes longiflora* A.Gray; 14870, 15079  
*Boerhaavia erecta* L.; 15405  
*Mirabilis albida* (Walter) Heimerl; 15035, 15352  
*Nyctaginia capitata* Choisy; 15081

## **OLEACEAE**

- Forestiera angustifolia* Torrey; 14906  
*Fraxinus pennsylvanica* Marshall; 15091  
*Menodora heterophylla* Moricand; 14943, 15523

## **ONAGRACEAE**

- Calylophus berlandieri* Spach; 14900, 15530  
*Gaura brachycarpa* Small; 14960, 15609  
*Gaura mckelveyae* (Munz) Raven & Gregory; 14866, 15541  
*Ludwigia peploides* (Kunth) Raven; 15613  
*Oenothera grandis* (Britton) Smyth; 14957, 15600  
*Oenothera speciosa* Nuttall; 15468, 15924

## **OXALIDACEAE**

- Oxalis dillenii* Jacquin; 14925, 15467

**PAPAVERACEAE**

*Argemone sanguinea* Greene; 14867, 15165

**PASSIFLORACEAE**

*Passiflora tenuiloba* Engelman; 15142

**PHYTOLACCACEAE**

*Rivina humilis* L.; 14965, P36370

**PLANTAGINACEAE**

*Plantago hookeriana* Fischer & Meyer; 14961, 15563,

*Plantago rhodosperma* Decaisne; 15524, 15509

*Plantago virginica* L.; 15499, 15602

**POLYGALACEAE**

*Polygala alba* Nuttall; 14974, 15536

**POLYGONACEAE**

*Polygonum pensylvanicum* L.; 15665

**PORTULACACEAE**

*Portulaca pilosa* L.; 15090

*Talinum aurantiacum* Engelman; 15145, 15735

*Talinum parviflorum* Nuttall; 15382

**PRIMULACEAE**

\**Anagallis arvensis* L.; P36475

**RANUNCULACEAE**

*Clematis drummondii* Torrey & Gray; 15010

**RHAMNACEAE**

*Colubrina texensis* (Torrey & Gray) A.Gray; 14882, 15577

*Condalia hookerii* M.C. Johnston; 14873, 15105

*Condalia spathulata* A.Gray; 15041, 15752

*Karwinskia humboldtiana* (J.A. Schultes) Zuccarini; 14875

*Ziziphus obtusifolia* (Torrey & Gray) A.Gray; 14913

## **RUBIACEAE**

*Diodia teres* Walter; 15213, 15591

*Galium aparine* L.; 15498

*Galium proliferum* A.Gray; 15503, 15636

*Houstonia croftiae* Britton & Rusby; 15616

*Houstonia micrantha* (Shinners) Terrell; 15598

*Richardia tricocca* (Torrey & Gray) Standley; 15088

## **RUTACEAE**

*Thamnosma texana* (A.Gray) Torrey; 15640

*Zanthoxylum fagara* (L.) Sargent; 15025

## **SALICACEAE**

*Populus deltoides* Marshall; 15133

*Salix nigra* Marshall; 15612

## **SAPINDACEAE**

*Sapindus saponaria* L.; 15753

## **SAPOTACEAE**

*Sideroxylon celastrinum* (Kunth) Pennington; P36378

## **SCROPHULARIACEAE**

*Agalinis strictifolia* (Benth) Pennell; 14985

*Bacopa rotundifolia* (Michaux) Wettstein; 15749

*Castilleja indivisa* Engelman; 15653

*Leucophyllum frutescens* (Berlandier) I.M. Johnston; 14939

*Nuttallanthus texanus* (Scheele) Sutton; 15169, 15486

*Veronica peregrina* L.; 15492

## **SIMAROUBACEAE**

*Castela erecta* Turpin subsp. *texana* (Torrey & Gray) J. Rose; 15756, P36473

## **SOLANACEAE**

*Chamaesaracha coniodes* (Moricand) Britton; 15400, 15474

*Lycium berlandieri* Dunal; 14921, 15194

*Physalis cinerascens* (Dunal) A. Hitchcock; 14932, 15102

*Solanum triquetrum* Cavanilles; 15013, 15608

**STERCULIACEAE**

*Hermannia texana* A.Gray; 14914

*Melochia tomentosa* L.; 15356

**ULMACEAE**

*Celtis laevigata* Willdenow; 15054

*Celtis pallida* Torrey; 14917, P36377

*Ulmus crassifolia* Nuttall; 15751

**URTICACEAE**

*Parietaria pensylvanica* Willdenow var. *obtusata* (Small) Shinnars;  
15172

*Urtica chamaedryoides* Pursh; 15482

**VERBENACEAE**

*Aloysia gratissima* (Gillies & Hooker) Troncoso; 15418

*Glandularia pumila* (Rydberg) Umber; P35307

*Glandularia quadrangulata* (Heller) Umber; 15003, 15569

*Lantana achyranthifolia* Desfontaines; 14952, 15512

*Lantana camara* L.; 14904, P35270

*Lippia graveolens* Kunth; 15157

*Phyla nodiflora* (L.) Greene; 14998, 15124

*Verbena halei* Small; 14899, 15466

*Verbena plicata* Greene; 15354

**VIOLACEAE**

*Hybanthus verticillatus* (Ortega) Baillon; 15533, 15629

**VISCACEAE**

*Phoradendron tomentosum* (DC.) A.Gray; 15040, P36368

**VITACEAE**

*Cissus incisa* Des Moulins; 14869, 15099

**ZYGOPHYLLACEAE**

*Guajacum angustifolium* Engelman; 14950

\**Tribulus terrestris* L.; 15076



## SEXUAL CHANGE IN *JUNIPERUS ARIZONICA*: FACULTATIVE MONECIOUS?

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### ABSTRACT

A two year study of the incidence of female cones on otherwise male trees of *Juniperus arizonica* revealed that about 5-10% of the trees had a few female cones interspersed with the male cones. Literature reports on sex change in *Juniperus* are reviewed. *Phytologia* 93(1): 43-50 (April 1, 2011).

**KEY WORDS:** *Juniperus arizonica*, monecious, dioecious, female cones, sexual expression.

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Vasek (1966), in a seminal study of the junipers of the western United States, tagged individual trees of *J. occidentalis* subsp. *australis* Vasek (now *J. grandis* R. P. Adams) and *J. osteosperma* (Torr.) Little and recorded their sexual expression for up to five years. Table 1 shows the results of his study. Although *J. o.* subsp. *australis* was reported as 90-95% dioecious (Vasek, table 4, 1966), some of the trees changed from male cones to producing both male and female cones (MBMBM, OMMB, MMMB, table 1) and some trees changed from female to none (FFFO, FOOO, table 1), but none changed from male to female or from female to male.

*Juniperus osteosperma* is about 85-90% monecious (Vasek, table 4). A few trees changed from female to both (FBBBB, FBBB, FBB, FFBB, table 1) and some changed from male to both (MBBB,

MMBB, table 1) and 2 trees changed in all combinations (FFMB, OMFB, Table 1). Thus, of the 84 trees tagged and followed, 3/43 *australis* and 7/41 *osteosperma* trees showed sex changes.

Table 1. Sexual expression of tagged plants in the San Bernardino Mtns. (*J. o. subsp. australis*) and White Mtns. (*J. osteosperma*). M = male cones only, F = female cones only, B = both male and female cones, O = no cones produced. Each letter represents an observation for one year. For example, OMFB means that the tree was observed 4 years with no cones (O) in the first year, male cones (M) in year 2, female cones (F) in year 3 and both male and female cones (B) in year 4. (data from Vasek, 1966)

<i>J. o. subsp. australis</i>				<i>J. osteosperma</i>			
# trees	pattern	# trees	pattern	# trees	pattern	# trees	pattern
1	MMMMM	4	FFF	11	BBBBB	1	FB BBB
5	MMMM	2	FF	15	BBB	3	FB BB
2	MMMO	1	BBB	7	BB	1	FBB
5	MMM	1	OOOO	2	FFFF	2	FFB
3	MM	1	MBMBM	1	FFF	2	M BBB
10	FFFF	1	OMMB	1	FFFO	1	M MB
5	FFFO	1	MMMB	1	MM	1	FFMB
1	FOOO			1	OOO	1	OMFB

It is also interesting that Vasek (1966) observed a female *australis* tree with a broken, forked branch. The lower (cambium intact) portion produced female cones and the upper (presumably stressed) portion produced male cones. This seems to imply that environmental conditions may play a role in sex expression.

Jordano (1991) reported on sex expression in *J. phoenicea* L., a species that is largely monocious. He found that strongly male trees did not convert to females (Table 2) and that the strongly female trees did occasionally convert to strong male trees (Table 2). One of the inconstant (inconsistent) male trees did have some female cones the next year. Jordano (1991) reported that strong males produced fewer

than 10 female cones, whereas inconstant males rarely exceeded 200 females cones. Strong female trees produced more than 100 cones, except in years of crop failures. In addition, he reported that male trees produced smaller female cones with fewer seeds and these female cones tend to be aborted before maturity (Jordano, 1991). He speculated that self-fertilized might be the cause of self-abortion.

Table 2. Gender expression in consecutive years of *J. phoenicea* (data from Jordano, 1991).

Gender in current year	Gender expression in the next year				
	Male	Incon-stant male	Mono-ecious	Incon-stant female	Female
Male	2	8	0	0	0
Inconstant male	6	17	1	1	0
Monecious	0	4	1	0	0
Inconstant female	0	0	1	0	1
Female	3	1	0	0	21

The purpose of the present study was to tag and examine the changes in sex expression in a population of *J. arizonica*.

## MATERIALS AND METHODS

Approximately 200 *J. arizonica* trees near Cottonwood, AZ were examined to determine if any male trees had female cones. Those male trees that produced some female cones were tagged and re-examined annually for the production of female cones.

## RESULTS AND DISCUSSION

In March, 2009, 18 male trees were found that had mature female cones (Table 3). In June, 2010, 3 additional male trees were discovered with female cones (Table 3, trees 19-21). Interestingly, none of the trees that bore new female cones in 2008 produced female cones (YF) in the winter of 2009 (Table 3). The mature fruit (MF) were from the 2008 pollination season (winter). Since none of the 21

trees had young fruit in March, 2009, then, of course, they would not have any mature fruit (MF) in June, 2010. Many of the trees that bore a few female cones in 2008 (YF, Table 3) did not bear any female cones in either 2009 or 2010. Two trees (#2 and 10) bore more, or about the same, number of female cones from 2008 to 2010 (but none in 2009).

Table 3. Sex expression in tagged male trees on successive years (2008, 2009, 2010). MF = mature fruit (1 yr old), YF = current year's fruit. No data is available for MF in 2008. YF in 2008 is based on MF found on the same tree in 2009.

Tree	2008	2009		2010	
	YF	YF	MF	YF	MF
1	50+	0	50	12	0
2	1+	0	1	9	0
3	3+	0	3	2	0
4	1000+	0	1000+	8	0
5	2+	0	2	0	0
6	11+	0	11	0	0
7	6+	0	6	0	0
8	1+	0	1	1	0
9	2+	0	2	0	0
10	10+	0	10	14	0
11	5+	0	5	0	0
12	5+	0	5	0	0
13	2+	0	2	0	0
14	13+	0	13	0	0
15	2+	0	2	0	0
16	9+	0	9	0	0
17	100+	0	100+	13	0
18	3+	0	3	0	0
19	?	0	?	5	0
20	?	0	?	0	0
21	?	0	?	1	0

Most of the male trees had only a few fruits (female cones), but trees #4 and 17 had at least 100 female cones (Table 3). Trees #1



and 4 that bore larger numbers of female cones (50, 100) in 2008 (YF, Table 3), had fewer fruits (12 and 8, respectively) in 2010 (Figure 1).



Figure 1. Single female cone surrounded by male cones on *J. arizonica*.

It might be noted that no strongly female trees with abundant female cones were found with male cones. However, seeing a few of the small male cones amongst the female cones is often very difficult.

In addition to counting the cones, in April, 2009, forked limbs were cut about 1/3 through the top portion (to mimic the broken branch noted by Vasek, 1966) on 25 male trees. In the spring of 2010, these cut branches were observed. All of these produced male cones on both the upper and lower forked branchlets. It seems reasonable that stressed branchlet (Vasek, 1966) might produce male cones if a species has that facultative ability. I (RPA) have observed that often one finds very few female cones on junipers in areas of severe drought.

However, floral sex ratios in monocious plants change in response to hormones: both auxins and gibberellins (Heslop-Harrison, 1972; Friedlander et al. 1977)

Freeman et al. (1981) reported that *J. osteosperma*, a monocious species, had a significantly higher frequency of male cones than female cones on trees in a xeric population (Table 4). It is interesting that *J. osteosperma* on the xeric site had slightly fewer trees (27.15%) with no cones (male or female)

Table 4. Comparison of the frequencies of male and female cones on terminal limbs (2 per tree), 25 trees per site of *J. osteosperma*, a monocious species.

Xeric site			Mesic site			F value for site-sex interaction
male	female	nothing	male	female	nothing	
54.85	17.50	27.15%	30.60	34.25	31.15%	30.57**

than on the mesic site (31.15%). Freeman et al. (1981) found a similar pattern with Gambel oak (*Quercus gambelii*) and black greasewood (*Sarcobatus vermiculatus*), the plants in mesic sites having a higher ratio of female flowers vs. male flowers. Freeman et al. (1981) concluded that there is a tendency for male flowers (cones) to be more prevalent in xeric sites and female flowers (cones) to be more prevalent in mesic sites.

Vasiliauskas and Aarssen (1992) examined the growth and special distribution of male and female *J. virginiana* trees. They reported that sex ratios were not related to age structure, stand density, or local competition intensity. However, they did find that male trees were taller than female trees and concluded that female trees pay a slightly greater cost for reproduction in terms of reduced vegetative growth.

However, Marion and Houle (1996) found no differences in radial growth patterns, annual elongation of the main axis, or size between male and female plants of *J. communis* var. *depressa* in a

north-south transect on the eastern coast of Hudson Bay. But they did report that the northern-most populations had a male-biased sex ratio in contrast to the southern-most populations that had a female-biased sex ratio. If the northern-most populations are under more environmental stress, then there appears to be an increase in males with more stressful environments.

Gehring and Whitham (1992) reported that, for *J. monosperma* Engelm., plants highly infested with mistletoe (*Phoradendron juniperinum* Engelm.) growing on a stressful (volcanic cinder, ash and lava) site, female plants were more highly infested than male plants. But on a less stressful site (sandy-loam), there was no significant difference between the infestation rates for females or males. Again, there does seem (at least in *J. monosperma*) to be some costs associated with berry (female cone) production under stressful conditions.

The present study merely focused on the production of a few female cones on otherwise male trees in the dioecious species *J. arizonica*. Is *J. arizonica* truly dioecious? It has been my (RPA) experience that one can find a few monecious individuals among thousands of trees examined for all the dioecious species of *Juniperus* (and nearly all species are dioecious). The presence of a few monecious individuals would not invalidate one saying that a given species is dioecious. In the present case of *J. arizonica*, it may be more correct to describe the species as dioecious, but rarely monecious.

The apparent ease with which male *J. arizonica* plants appear to produce a few female cones seems to indicate the dioecious/monecious mode is somewhat porous and may be easy to bridge. Could *J. arizonica* have the facultative ability to produce viable seed from 'male' trees to aid in colonization by long distance dispersal? If only a few male tree seeds are dispersed (by chance), then it could be advantageous to produce some seed by a 'partially' monoecious plant(s) to start a new population. Of course, we do not yet know if the seeds produced on the 'male' plants in this study are viable (Adams and Thornburg, in progress).



Sex changes and conversion from dioecious to monocious *Juniperus* plants (Vasek, 1966; Jordano, 1991, this study) raise some evolutionary questions that deserve a closer look in the future.

### ACKNOWLEDGEMENTS

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## CHEMOSYSTEMATICS OF *JUNIPERUS*: EFFECTS OF LEAF DRYING ON ESSENTIAL OIL COMPOSITION II

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### ABSTRACT

The essential oils of leaves of *J. virginiana* tree were collected and analyzed as fresh vs. air dried and stored at ambient conditions (21° C) for up to 16 months before extraction. ANOVA of the 58 components revealed 4 significant and 19 highly significant differences among the 8 sample sets, with the major changes occurring between 8 and 16 months storage. PCO of the samples showed the 16 mo. samples to be clearly clustered. In contrast to the previous 8 mo. study (Adams, 2010), unexpected changes in the oils raise concerns about mixing analyses of oils from fresh, recently dried and 16 mo. stored leaves of *Juniperus* for chemosystematic studies. *Phytologia* 93(1)51-62 (April 1, 2011).

**KEY WORDS:** *Juniperus*, oils from dried leaves, storage tests, chemosystematics.

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In a previous study (Adams, 2010), leaves of *Juniperus pinchotii* Sudw. and *J. virginiana* L. were air dried (as per specimens) and analyzed as fresh and stored (ambient lab conditions, 21° C) for up to 8 months before extraction. The leaf oils of both species proved to be remarkably stable. For *J. virginiana*, ANOVA of the 58 components revealed only 9 significant and 4 highly significant differences among the 7 sample sets. PCO of the samples showed some clustering by length of storage, but with considerable intermixing of samples.

Achak et al. (2008, 2009) compared the leaf essential oils from fresh and air dried (22° C, 16 days) leaves of *J. thurifera* L., *J. phoenicea* L. and *J. oxycedrus* L. and found only small differences.

The purpose of the present study is to report on changes in the composition of the steam distilled leaf oil of *J. virginiana* from specimens stored for 16 months.

## MATERIALS AND METHODS

**Plant material** - *J. virginiana*, Adams11768, cultivated, nw corner of Gruver City Park, Hansford Co. TX, initial bulk collection: 23 Apr 2009. Voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

**Isolation of oils** - Fresh (100 g.) and air dried (10-15 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields.

**Analyses** - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. For the comparison of oils obtained from leaves stored for various periods, associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967). Principle

Components Analysis (PCA) as formulated by Veldman (1967) was performed to examine correlations between components.

## RESULTS AND DISCUSSION

Table 1 shows the composition of the leaf oil of *J. virginiana* and a comparison of components over the 16 month storage period. Perhaps most non-intuitive is that the percent oil yield did not decline (significantly) throughout the 16 month study (Table 1). It would seem that the loss of volatiles from dry leaves over such a period would be significant. Shanjani et al. (2010) reported that  $\alpha$ -pinene (the major and most volatile component) declined from 23.9 to 14.2% when the foliage of *J. excelsa* was air dried. Achak et al. (2008) found oil yields to be greater from fresh than air dried leaves from 2 populations of *J. thurifera* var. *africana*, but with a lower yield in another population. Later, Achak et al. (2009) reported lower oil yields in dried leaves of *J. thurifera* var. *africana* and *J. oxycedrus*, but a much higher yield from dried leaves of *J. phoenicea*.

The compounds (as percent total oil) are remarkably stable during the drying and storage tests for the first 8 months but there are major changes between 8 and 16 months storage tests. In the tests up to 8 months storage, only 9 compounds significantly differed, and only 4 compounds differed highly significantly (Adams, 2010). However, distillation of leaves stored for 16 months revealed 4 significant and 19 highly significant differences (Table 1). Several compounds had major declines in concentration from 8 to 16 month: sabinene (17.6, 13.3), limonene (14.6, 11.7),  $\beta$ -phellandrene (9.7, 8.0) and germacrene D-4-ol (3.8, 3.4). In contrast, several compounds increased: safrole (9.9, 11.1), methyl eugenol (2.2, 2.5), elemol (5.8, 8.8) and 8- $\alpha$ -acetoxyelemol (10.7, 12.4). Figure 1A shows the major compounds that declined. Notice that sabinene, limonene, and  $\beta$ -phellandrene show similar patterns. Pregeijerene B shows a gradual decline from 1 month to 16 months.

The patterns for 4 of the major components that increased during the study are shown in figure 1B. Safrole and methyl eugenol

(both from the phenyl propanoid pathway) show similar patterns along with elemol. However, 8- $\alpha$ -acetoxyelemol (dashed line, Fig. 1B) increased from fresh to week 1, then declined, then increased to 2 month, then declined and finally increased in the final, 16 month, sample.

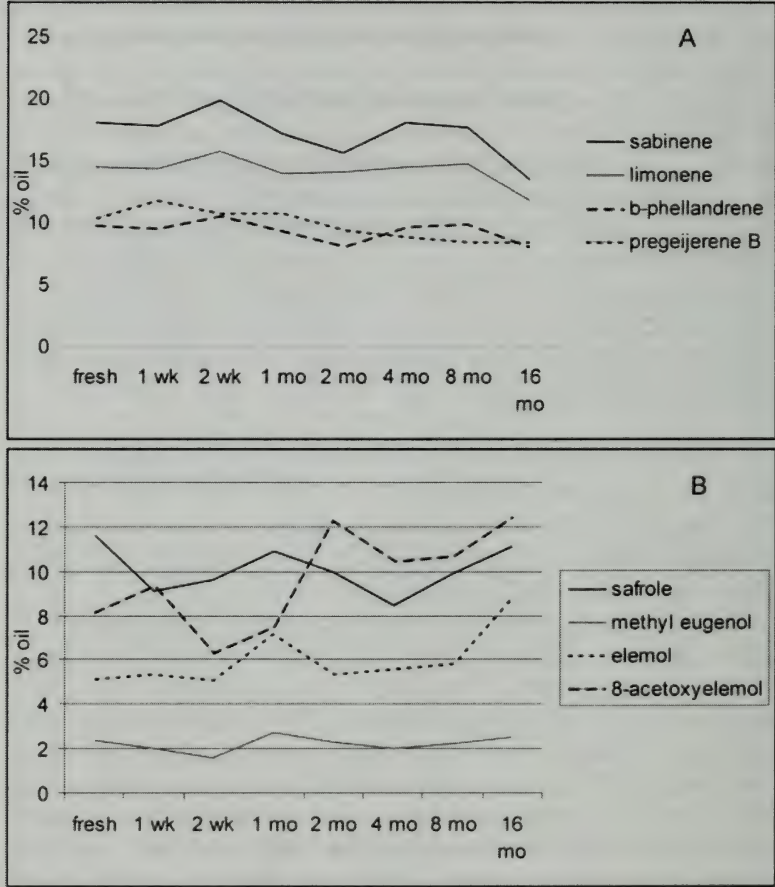


Figure 1. A (upper) Changes in concentration (% total oil) for four major components that declined during leaf storage. B (lower) Changes in concentration (% total oil) for four major components that increased during leaf storage.



The leaf essential oils in *Juniperus* are stored in leaf glands. In *J. virginiana*, the leaf glands are generally not ruptured and often sunken beneath the waxy cuticle. So volatilization in this instance seems to be minimized by the intact glands and waxy cuticle.

To estimate the impact of the utilization of oils from fresh versus dried and stored leaves, principal coordinates analysis (PCO) was performed. The PCO (figure 2) shows the major trend is for the separation of the 16 months samples on axis 1 (21% of the variance among samples).

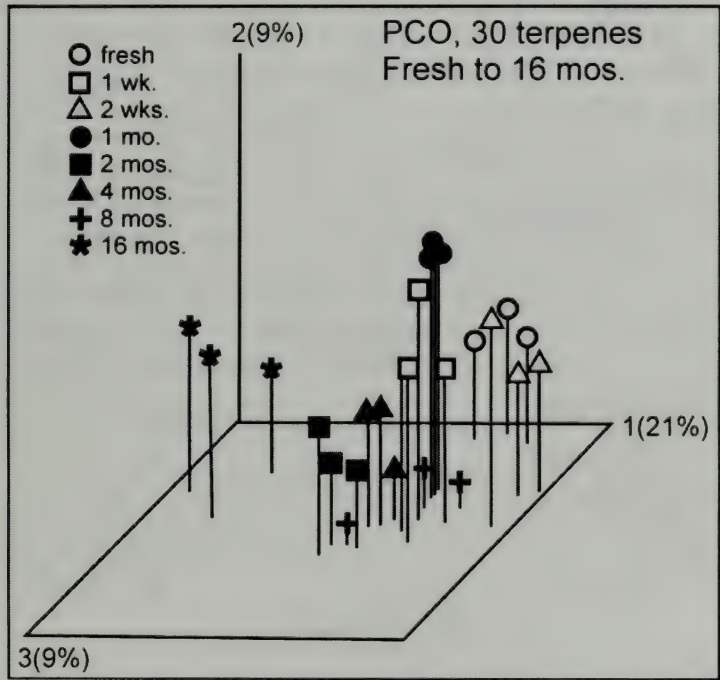


Figure 1. PCO of 8 sample sets ranging from fresh to storage for 16 months at ambient herbarium conditions (air conditioned, 21°C).

Examination of the correlation between components was performed by PCA. Factoring the correlation matrix resulted in eigenroots that appeared to asymptote after 5 eigenroots. These accounted for 44.2, 11.3, 9.39, 6.64 and 6.45% of the variance among the components. PCA shows correlation patterns among various classes of terpenoids and phenolic compounds (Fig. 2). In general, the

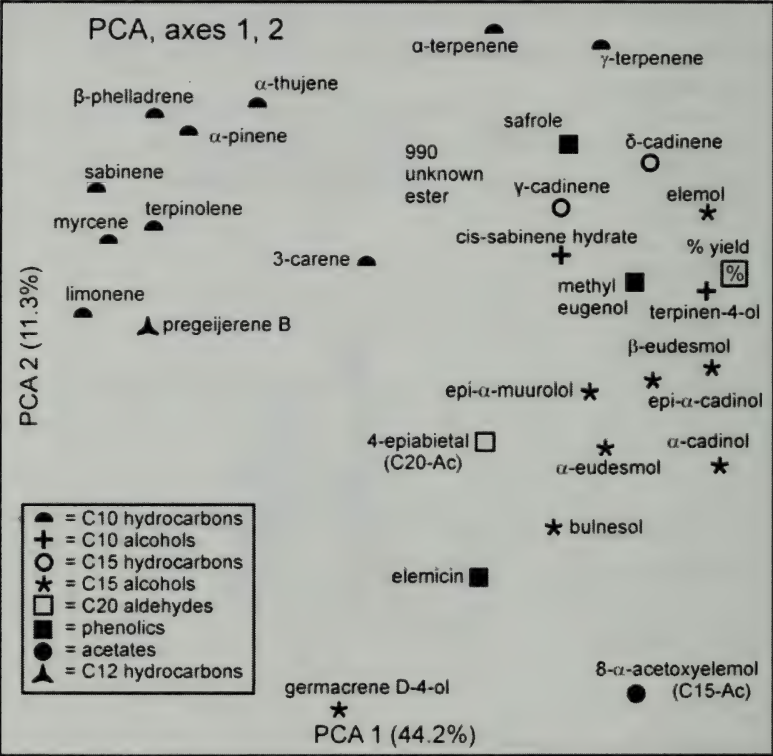


Figure 2. PCA of 39 components from *J. virginiana* samples stored from fresh to 16 months.

hydrocarbons are correlated (upper left, Fig. 2) and the sesquiterpene alcohols are clustered (middle right, Fig. 2). The phenolics (safrole, elemicin, methyl eugenol), from the phenyl propanoid pathway, are

somewhat scattered (Fig. 2). It appears that axis one is also separating components that increased (phenolics, sesquiterpene alcohols) from those that decreased (terpene hydrocarbons) during the 16 month study. It should be noted that only the first 2 axes are displayed, so separation of variables on the 3rd and succeeding axes is not accounted for in figure 2.

## CONCLUSIONS

In this study, ANOVA revealed 4 significant and 19 highly significant differences among the 8 sample sets, with the major changes occurring between 8 and 16 months storage. PCO of the samples showed the 16 mo. samples to be clearly clustered. In contrast to the previous 8 mo. study (Adams, 2010), unexpected changes in the oils raise concerns about mixing analyses of oils from fresh, recently dried and 16 mo. stored leaves of *Juniperus* for chemosystematic studies. If such studies were conducted among species with large differences in the essential oil compositions, the utilization of oils from both fresh and air dried leaves might still be valid. However, the present study raises concerns about the unexpected changes between 8 and 16 months of herbarium storage. It may be difficult to predict the stability of leaf essential oils in specimens over long periods of storage.

## ACKNOWLEDGEMENTS

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Table 1. Comparison of the composition of leaf oils from fresh leaves of *J. virginiana* vs. leaves dried and stored at 21° C. F sig = F ratio significance, P=0.05 = \*; P=0.01 = \*\*; ns = non significant, nt = not tested.

Al	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	18mo	F sig
	percent yield	0.55	0.52	0.48	0.51	0.48	0.56	0.53	0.55	ns
924	α-thujene	0.4	0.4	0.5	0.5	0.4	0.4	0.5	0.4	ns
932	α-pinene	0.7	0.7	0.9	0.7	0.5	0.6	0.8	0.5	**
945	α-fenchene	t	t	t	t	t	t	t	t	nt
969	sabinene	18.0	17.7	19.8	17.1	15.5	17.9	17.6	13.3	**
974	β-pinene	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.2	ns
988	myrcene	1.2	0.9	1.1	0.8	0.7	0.7	0.5	0.2	**
990	<u>74,87,43,115</u>	0.5	0.3	0.4	0.3	0.4	0.3	0.4	0.3	**
1008	3-carene	0.6	0.6	0.6	0.5	0.5	0.7	0.9	0.4	**
1014	α-terpinene	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.4	nt
1024	limonene	14.4	14.2	15.6	13.8	14.0	14.4	14.6	11.7	**
1025	β-phellandrene	9.6	9.3	10.4	9.2	7.9	9.5	9.7	8.0	**
1054	γ-terpinene	0.6	0.5	0.5	0.6	0.5	0.6	0.5	0.6	ns
1065	cis-sabinene hydrate	0.5	0.5	0.5	0.5	0.6	0.6	0.5	0.6	ns
1086	terpinolene	0.8	0.7	0.8	0.7	0.7	0.8	0.7	0.5	*
1096	trans-sabinene hydrate	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.3	ns
1097	linalool	0.4	0.3	0.6	0.5	0.5	0.7	0.5	1.0	ns
1100	n-nonanal	t	t	0.2	t	0.2	t	t	t	ns
1118	cis-p-menth-2-en-1-ol	t	t	t	t	t	0.2	t	t	nt

Al	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	16 mo	F sig
1136	trans- p-menth-2-en-1-ol	t	t	t	t	t	t	t	t	nt
1148	citronellal	0.2	t	t	t	t	t	t	t	nt
1174	terpinen-4-ol	1.3	0.8	0.8	0.9	1.1	1.2	0.9	1.5	**
1186	$\alpha$ -terpineol	t	t	t	t	t	t	t	t	nt
1195	methyl chavicol	0.1	0.2	t	0.2	0.2	0.2	t	t	ns
1223	citronellol	0.2	t	t	t	0.2	0.2	t	t	ns
1261	152,123,81,77, aromatic	0.4	0.4	0.3	0.4	0.3	0.4	0.3	0.3	ns
1274	pregeijerene B	10.2	11.7	10.7	10.6	9.4	8.7	8.3	8.2	**
1285	safrrole	11.6	9.1	9.6	10.9	10.0	8.5	9.9	11.1	**
1322	methyl geranate	0.1	t	t	t	0.1	0.1	t	t	nt
1350	citronellyl acetate	t	t	t	t	t	t	t	t	nt
1379	geranyl acetate	t	t	t	t	t	t	t	t	nt
1403	methyl eugenol	2.4	2.0	1.6	2.7	2.3	2.0	2.2	2.5	**
1417	(E)-caryophyllene	t	t	t	t	t	t	t	t	nt
1447	43,105,149,178, aromatic	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	ns
1465	cis-murola-4(14),5-diene	t	t	t	t	t	0.2	t	0.2	nt
1491	epi-cubebol	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	ns
1500	$\alpha$ -muurolene	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.3	ns
1513	$\gamma$ -cadinene	0.3	0.4	0.5	0.6	0.5	0.5	0.4	0.5	*
1522	$\delta$ -cadinene	0.8	0.7	0.8	1.0	0.8	0.9	0.9	1.0	**

Al	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	16 mo	F sig
1539	$\alpha$ -copaen-11-ol	t	0.3	t	t	t	t	t	t	nt
1548	elemol	5.1	5.3	5.1	7.2	5.4	5.5	5.8	8.8	**
1555	elemicin	0.8	0.8	0.5	0.8	0.9	0.7	1.1	0.7	ns
1565	(3Z)-hexenyl benzoate	0.2	t	0.2	0.2	0.3	0.2	t	t	ns
1574	germacrene-D-4-ol	2.8	3.4	3.4	2.6	3.5	3.0	3.8	3.4	**
1630	$\gamma$ -eudesmol	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.2	ns
1638	epi- $\alpha$ -cadinol	0.6	0.6	0.5	0.6	0.6	0.6	0.6	0.9	**
1638	epi- $\alpha$ -muurolol	0.6	0.6	0.5	0.7	0.6	0.6	0.7	0.8	*
1649	$\beta$ -eudesmol	0.4	0.5	0.4	0.5	0.2	0.6	0.6	0.7	**
1652	$\alpha$ -eudesmol	0.6	0.7	0.6	0.6	0.7	0.7	0.8	0.9	ns
1652	$\alpha$ -cadinol	1.0	1.0	0.8	1.0	1.0	1.1	1.2	1.4	**
1670	bulnesol	0.5	0.4	0.4	0.3	0.5	0.5	0.6	0.5	*
1688	shyobunol	t	t	t	t	0.2	0.2	t	t	ns
1746	8- $\alpha$ -11-elemodiol	t	t	0.2	t	0.3	0.4	0.3	t	ns
1761	iso to 8- $\alpha$ -acetoxyelemol	0.2	0.3	0.2	0.2	0.3	0.3	0.3	0.3	ns
1792	8- $\alpha$ -acetoxyelemol	8.1	9.3	6.3	7.5	12.3	10.5	10.7	12.4	**
2054	41,81,137,270,	0.2	0.2	t	0.3	0.3	0.3	0.3	0.4	nt
2087	abietadiene	t	t	t	t	t	t	t	t	nt
2298	4-epi-abietal	0.4	0.3	0.3	0.2	0.4	0.4	0.3	0.5	**

AI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	16 mo	F sig
2312	abiet-7,13-dien-3-one	t	t	t	t	t	t	t	0.1	nt

AI = Arithmetic Index on DB-5 column (see Adams, 2007). Unidentified compounds have the major ions listed. The first ion (underlined) is the base (100%) ion. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.



**KEYS TO THE FLORA OF FLORIDA -- 27,  
*FRAXINUS* (OLEACEAE)**

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**ABSTRACT**

*Fraxinus* (Oleaceae) is represented in Florida by 4 species. All are native; none is endemic. Habitat preferences are noted. The specific independence of *F. profunda* is supported. Variability of *F. caroliniana* is addressed, with recognition of var. *caroliniana*, var. *cubensis*, and var. *pauciflora*, comb. nov. An amplified key is given to the Florida taxa. *Phytologia* 93(1):63-72 (April 1, 2011).

**KEY WORDS:** *Fraxinus*, Oleaceae, Florida flora.

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One might assume that within a genus such as *Fraxinus* (Oleaceae), the ashes, trees that are so familiar to so many, for so many years, and so important a component of the forest flora, there would remain no secrets or disagreements as to a simple matter of taxonomy: how many species are there in Florida? Not so! A much-followed floristic summary of the state by R. P. Wunderlin & B. F. Hansen (Guide to Vasc. Plants of Florida. 2003) admitted only 3 species. The only modern monograph of North American species, by Gertrude N. Miller (Agric. Exp. Sta., Ithaca, New York, Mem. 335. 1955), recognized 4 species within Florida, as did a careful nomenclatural compendium of American trees by E. L. Little (Checklist U.S. Trees, U.S. Dept. Agric. Handb. 541. 1979), and a detailed description of southeastern woody plants by R. K. Godfrey (Trees, Shrubs and Woody Vines. 1988). Some previous authors, J. K. Small (1903, 1933) and H. Kurz & R. K. Godfrey (Trees of Northern Florida. 1962) have distinguished 5 species within the state. Most recently, G. L. Nesom (pers. comm.) is preparing text for *Flora North America* that will delineate 6 species native to Florida. [Other significant studies: A key

to all U.S. species, by W. A. Dayton (J. Wash. Acad. Sci. 44: 385-390. 1954); a synopsis of southeastern species, by K. A. Wilson & C. E. Wood (J. Arnold Arbor. 40: 371-375. 1959); SEM leaf surface features, by J. W. Hardin & R. L. Beckmann (Brittonia 34: 129-140. 1982).]

The present treatment is atypical of this series in addressing so few species, and where photographs and keys to their identification are so readily available. But it is imperative that these important trees be understood as to the number of species that occur in the state and the characteristics by which they may be identified. And the perplexing variation within the *Fraxinus caroliniana* complex needs to be addressed. Long familiarity with the Florida ashes suggests that at least some of the remaining ambiguities may be resolved.

*Fraxinus americana* L., the White Ash, presents no problems, either in identification or nomenclature. It is quickly recognized by its samaras with chubby fruit-bodies and leaves that are whitish beneath. Even a leaf fragment can be identified without question: the whitish undersurface is closely set by discrete areas of minute rough-textured papillae, visible at 100X (Hardin & Beckmann, 1982). [Hardin & Beckmann reject "papillae," preferring "coronulate" as a more precise term.]

The slightly differing "Biltmore Ash," *Fraxinus biltmoreana* Beadle (= *F. americana* var. *biltmoreana* (Beadle) J. Wright ex Fern.), distinguished by its tomentulose leaves and twigs, seems not reliably reported south of Georgia.

*Fraxinus pennsylvanica* Marsh., the Green Ash ("Red Ash"), and *Fraxinus profunda* (Bush) Bush in Britt., the Pumpkin Ash, are closely related, but readily distinguished when samaras are present. The leaves, too, differ in size and pubescence, although not always convincingly so.

Though *Fraxinus profunda* clearly is a close relative of *F. pennsylvanica*, it is distinguished by a series of "gigas" characteristics. Miller (1955: 45-47) has tabulated 13 differences between the two

species, some subtle, others quite apparent. (Those most useful are employed in the accompanying key.) Differences between the Pumpkin Ash and the Green Ash have also been well described and illustrated by M. L. Fernald (Rhodora 40: 450-453, plates 528, 529. 1938). That these two taxa fully merit specific rank is attested to by most dendrologists with North Florida experience (Small, 1933; Fernald, 1950; Gleason, 1952; Miller, 1955; West & Arnold, 1956; Kurz & Godfrey, 1962; Little, 1979; Godfrey & Wooten, 1981; Clewell, 1985; Nesom, 2010; contra, Wunderlin, 1998). No justification for merger has been seen.

The origin of *Fraxinus profunda* has long stimulated debate and conjecture. One schooled in introductory genetics will not think it possible that a species may receive one-third of its genetic component from one parent and two-thirds from the other. Yet this ratio may describe the origin of the Pumpkin Ash. J. W. Wright (Morris Arbor. Bull. 8: 33-34. 1957; U.S. Dept. Agric. Handb. 271. 1965) has interpreted the Pumpkin Ash as a stable hexaploid derived from a diploid Green Ash and a tetraploid White Ash. The apparent discontinuous distribution of Pumpkin Ash, mapped as occurring separately in the upper Mississippi valley, the coastal Carolinas, and northern Florida (E. L. Little, U.S. Dept. Agric. misc. publ. 1342. 1977), has given credence to the suggestion that different populations of the Pumpkin Ash may have had independent origins. More detailed mapping (Nesom, Phytoneuron 2010-21: 1-6) shows a near-contiguous distribution of Pumpkin Ash from Maryland into South Carolina, a hiatus across Georgia, many stations in northern Florida, few in Alabama and eastern Mississippi, and an abundance from Louisiana northward [A Green Ash/White Ash origin, however, seems not to be supported by SEM epidermal structures (Hardin & Beckmann, 1982).]

The name *Fraxinus tomentosa* Michx. f., although earlier than the name *Fraxinus profunda* (1813 vs. 1897), has been demonstrated to be nomenclaturally superfluous (E. L. Little, J. Wash. Acad. Sci. 42: 369-380. 1952), and is thus illegitimate.

*Fraxinus caroliniana* Mill., the Pop Ash ("Carolina Ash"), is the only native ash that extends into the southern peninsula. Its often multi-trunked habit may permit easy recognition in the field even in the absence of its elliptic-winged samaras. But variability of the samaras fosters confusion in herbarium materials with *F. profunda*, while at times variability of the leaves suggests intergradation with *F. pennsylvanica*. [This variability is here treated as falling within three varieties.]

Habitats preferred by these four species are largely distinct. *Fraxinus americana*, as is widely recognized, is a species of mesic soils. *Fraxinus pennsylvanica* is found on lower sites, also mesic or sometimes seasonally swampy. *Fraxinus profunda* is truly hydric, restricted to stream-bottom swamps, especially those fed by springs. And *Fraxinus caroliniana* (with its varieties) grows on stream banks and among cypress (notably in the Fakahatchee Slough of Collier County, where it is often the dominant understory tree, well encrusted with epiphytic orchids and ferns).

The fidelity with which each of these species adheres to its preferred habitat is shown by a transect across the shallow valley of the Santa Fe River, a major tributary of the Suwannee River in the north-central Florida peninsula. [A convenient viewing point might be near Hornsby Run, a spring-fed stream north of High Springs, northwest corner of Alachua County.] The wooded uplands are of oaks, pignut hickory, sweetgum, Florida maple and other typical "hammock" trees of the upper peninsula. White Ash is present, although not in great numbers. Well down the slope toward the river the White Ash thins out and Green Ash appears; though the two species may at times occur together, the topographic gradient serves as a rather effective predictor of which species to anticipate. Small several-trunked trees of (typical) Pop Ash are scattered along the river's edge, never intruding among the Green Ash only slightly up-slope. And Pumpkin Ash is exclusively on the always-saturated muck soils of the wooded swamp bordering the spring run, with red maple and cypress. Though they are at times found only short distances from one another, the Pop Ash is not present deep within the spring-run swamps, nor the Pumpkin Ash on the



intermittently flooded-and-dry river banks. [*F. caroliniana* var. *pauciflora* occurs on muck soil of swamps and oft-submerged lake shores, and could perhaps be added to this transect.]

Maximum sizes of Florida ashes have been documented by a Florida champion tree survey (D. B. Ward & R. T. Ing, Big Trees, the Florida Register. 1997). Maximum heights recorded: Pumpkin Ash, to 100 ft.; White Ash, to 95 ft.; Green Ash, to 93 ft.; and Pop Ash, to 58 ft. Maximum circumferences recorded: White Ash, to 169 in.; Green Ash, to 123 in.; Pumpkin Ash, to 119 in.; and Pop Ash, to 56 in. Thus *Fraxinus americana*, *F. pennsylvanica*, and *F. profunda* are large trees, similar to one another in height and circumference (with *F. americana* perhaps of somewhat greater girth), while *F. caroliniana* is in both respects much the smaller.

From time to time individuals have been encountered that are intermediate in one or more characters between the recognized species of *Fraxinus*. Miller (1955) noted collections from North Carolina, seemingly of hybrids between the Pumpkin Ash and (apparently) White Ash. She found trees in New York that indicated an "interaction" between Pumpkin Ash and Green Ash. She (p. 25) discussed an early report (Sargent, 1902) of trees in Lake County [central peninsular] Florida that she tentatively interpreted as hybrids between *F. caroliniana* and *F. americana* (now identified as *F. caroliniana* var. *pauciflora*; see below). Trees that could confidently be identified as interspecific hybrids were not encountered in the present study. Hybridity, though it may well be present, is not considered a significant source of variability among the Florida ashes.

*Fraxinus caroliniana*, within itself, is the most variable of the Florida ashes. M. L. Fernald & B. G. Schubert (Rhodora 50: 186-190. 1948) and Fernald (Manual. 1950) attempted to understand the complex by describing 3 varieties: var. *caroliniana*, with samaras broadly oblong-ob lanceolate to sub-elliptic, 1-2 cm. broad and 2-5-4.5 cm. long; var. *oblanceolata* (M. A. Curtis) Fern. & Schub., with samaras oblanceolate, 1.0-1.3 cm. broad and 3.5-5.5 cm. long; and var. *cubensis*

(Griseb.) Lingelsh., with samaras narrowly oblanceolate, 0.5-0.9 cm. broad and 3-5 cm. long. All were stated to occur in Florida.

These varieties of *Fraxinus caroliniana*, as delineated by Fernald & Schubert (1948), have not been recognized by later authors. Miller (1955) merely appended Fernald & Schubert's names to her synonymy, without reference in her text. Godfrey (1988: 511) did not go beyond noting the fruits of Pop Ash to be "very variable from plant to plant."

Though the taxa delineated by Fernald & Schubert are not acknowledged here, the present study, as well as a careful analysis by G. Nesom (Phytoneuron 2010-39: 1-13), suggests that recognition of entities within the *Fraxinus caroliniana* complex does permit assignment of nearly all individuals to a named taxon. Nesom argued that the complex consists of three entities, each of specific rank: *F. caroliniana* s.s., *F. cubensis*, and *F. pauciflora*. His *F. caroliniana* corresponds quite exactly with materials from the Carolinas, the type locality of the species. Nesom's *F. cubensis*, limited by him to central and south Florida (Fernald, 1950, suggested it reached Virginia), is readily recognized, at least in the southern peninsula, where in the absence of other ashes its samaras are quite consistent in size and form. Nesom strikes a chord of originality in recognizing Nuttall's long-slighted *F. pauciflora*, not only as the single Lake County population that had puzzled Miller (above), but as a frequent to common small tree with roughly the same North Florida range as his *F. caroliniana*.

Nesom's analysis goes far toward explaining the variation so long bedeviling *Fraxinus caroliniana*. It relies, however, on a character that cannot be seen with the naked eye, and indeed is scarcely visible at any magnification below 100X. This is the presence on the lower leaf surface of specimens he identified as *F. pauciflora* of a "micro-foveolate-papillose" appearance, with a "cuticular reticulum overlaying and obscuring the epidermal surface." In contrast, specimens he identified as *F. caroliniana* s.s. and *F. cubensis* lacked such an overlay and permitted stomata and other surface features to be seen.

In the field a quite different character has utility. This is the single-trunked habit of Nesom's *F. pauciflora*, and multi-trunked, often almost shrubby habit of *F. caroliniana* s.s. (Nesom, pers. comm., Aug 2010).

With this recognition of a population that perhaps may be reliably identified by the leaf surface character supported by the habit differences, and previous recognition of the South Florida population known as *F. cubensis*, as well as typical *F. caroliniana*, one is obligated to acknowledge these taxa at some level. The long history of competent observers having failed to note these differences is evidence that they are unremarkable, to say the least. The rank of species, as employed by Nesom, is believed to diminish the degree of clarity usually expected at such rank. Here, the rank of variety is seen as sufficient. Thus, the needed new combination is formed.

***Fraxinus caroliniana* Miller var. *pauciflora* (Nuttall) D. B. Ward, comb. et stat. nov.** Basionym: *Fraxinus pauciflora* Nuttall, N. Amer. Sylva 3: 61. 1849. TYPE: U.S.A. Georgia [Charlton Co.]: "in the neighborhood of Trader's Hill," William Baldwin s.n., no date (holotype: PH - fide Nesom, 2010).

The morphological differences believed most useful in separating the Florida species and varieties of *Fraxinus* are detailed in the accompanying key.

**FRAXINUS L.** Ashes <sup>1</sup>

1. Leaflets whitish beneath (the surface with small islets of minute white papillae, visible at 25X); leaf-scars deeply concave along upper edge; samaras with wing decurrent only on distal 1/3 of fruit-body; wing 5-10 mm. wide; fruit-body short and plump, 6-10 mm. long (length <8 times width), markedly thicker than adjacent wing. Large tree, to 30 m. Well-drained mesic hammocks. North Florida, south to upper peninsula (Citrus, Marion counties); frequent (rare or absent in w. panhandle and n.e. peninsula). Spring. [*Fraxinus Smallii* Britt.]

WHITE ASH.

***Fraxinus americana* L.**

1. Leaflets pale to medium green beneath (without minute papillae); leaf-scars slightly convex to concave along upper edge; samaras with wing decurrent to middle of fruit-body or beyond; fruit-body elongate and slender (length >10 times width), not appreciably thicker than surrounding wing.

2. Samaras 7-20 mm. wide, broadly elliptic to narrowly oblanceolate or spatulate; fruit-body 12-18 mm. long, its lateral edges indistinct; leaflets usually 5 (infreq. 3 or 7), 1.8-4.0 cm. broad, thin textured, glabrous beneath, variable in shape: broadly ovate, obtuse, serrate, to narrowly ovate, acute, entire; petiolules <1.0 cm. long. Shrub to mid-sized tree, to 20 m., often multi-trunked from base. Lake margins, river swamps. Spring.

POP ASH.

***Fraxinus caroliniana* Mill.**

- a. Samaras broadly elliptic to suborbicular (widest at midpoint), 10-20 mm. wide, lateral veins (each side) 12-20, the apex rounded; fruit-body >1/2 length of samara. Shrub or small multi-trunked tree. Panhandle (Escambia Co.), east through north Florida, south to upper peninsula (Levy, Alachua, Flagler counties); frequent. [Incl. var. *oblanceolata* (M. A. Curtis) Fern. & Schub.]

POP ASH (typical).

**var. *caroliniana***

- a. Samaras oblanceolate or spatulate (widest above midpoint), 6-12 mm. wide, lateral veins (each side) 8-12, the apex rounded or acute; fruit-body  $\pm$  1/2 length of samara.



- b. Lower surface of leaflets with a "cuticular reticulum," stomates obscured (at 100X); samaras oblanceolate (apex usually acute), 8-12 mm. wide. Small single-trunked tree. Panhandle, across north Florida, south to mid-peninsula (Orange, Osceola counties); common (infrequent in w. panhandle). [*Fraxinus pauciflora* Nutt.]

WATER ASH. var. **pauciflora** (Nutt.) D.B.Ward

- b. Lower surface of leaflets clear, stomates visible (at 100X); samaras narrowly oblanceolate to spatulate (apex often rounded), 6-10 mm. wide. Mid-sized multi-trunked tree. Upper peninsula (Citrus, Marion counties), to south peninsula (Collier, Monroe counties); frequent, at places (Fakahatchee) the dominant understory. The only native ash south of mid-peninsula. [*Fraxinus cubensis* Griseb.; *Fraxinus caroliniana* ssp. *cubensis* (Griseb.) Borhidi.]

CUBAN POP ASH. var. **cubensis** (Griseb.) Lingelsh.

2. Samaras 4-12 mm. wide, rhombic to narrowly spatulate or linear; fruit-body visibly distinct, slightly thicker than surrounding wing; leaflets usually 7 (infreq. 5), of uniform shape: broadly lanceolate, firm textured, acute to acuminate, entire.

3. Samaras 40-60 mm. long, narrowly rhombic to spatulate, 7-12 mm. wide, the upper margins straight or gently curved, apically rounded to truncate or emarginate; fruit-body 14-20 mm. long; leaflets 2.5-4.0 cm. broad, usually with a fringe of soft white hairs along sides of midvein beneath; petiolules mostly 1.2-2.0 cm. long. Large tree, to 30 m. Swamps, hydric hammocks, most often along spring-fed streams. Panhandle and north peninsula (south to Marion Co.); infrequent. Spring. [*Fraxinus pennsylvanica* var. *profunda* (Bush) Sudw.; *Fraxinus tomentosa* Michx. f.]

PUMPKIN ASH. **Fraxinus profunda** (Bush) Bush in Britt.

3. Samaras 30-40 mm. long, nearly linear, 4-8 mm. wide, the upper margins straight, apically rounded to acute; fruit-body 8-16 mm. long; leaflets 1.8-3.0 cm. broad, glabrous or with scattered fine hairs beneath; petiolules mostly 0.5-1.5 cm. long. Large tree, to 30 m. Low mesic hammocks. Panhandle to upper peninsula (Citrus, Sumter, Brevard counties); infrequent. Spring.

GREEN ASH.

***Fraxinus pennsylvanica* Marsh.**

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<sup>1</sup> This paper is a continuation of a series begun in 1977. The "amplified key" format employed here is designed to present in compact form the basic morphological framework of a conventional dichotomous key, as well as data on habitat, range, and frequency. Amplified keys are being prepared for all genera of the Florida vascular flora; the present series is restricted to genera where a new combination is required or a special situation merits extended discussion.

*Fraxinus* has long been of interest to Florida botanists. Florida specimens were carefully examined in 1964 by Susan Elam Ruiz (FLAS), then in 1979 by Walter S. Judd (FLAS); their measurements and annotations have been of value to the present study. My long-time friend, Robert Godfrey (FSU), agonized with me over the characteristics separating the different taxa (as well as their number, as witnessed by his recognition of 5 species in 1962, reduced to 4 in 1988). Guy L. Nesom and I have repeatedly exchanged copies of our *Fraxinus* manuscripts, to my benefit.

**TAXONOMY OF INFRASPECIFIC TAXA OF *ABIES*  
*LASIOCARPA*: LEAF ESSENTIAL OILS AND DNA OF *ABIES*  
*LASIOCARPA*, VAR. *BIFOLIA* AND VAR. *ARIZONICA*.**

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**ABSTRACT**

Detailed analyses of the leaf essential oils of *Abies lasiocarpa* var. *lasiocarpa* (Olympic Peninsula, WA), *A. l.* var. *bifolia* (MT, UT) and *A. l.* var. *arizonica* (AZ) is presented to update the analyses of Hunt and von Rudloff (1979). The coastal alpine fir (*A. l.* var. *lasiocarpa*) is very strongly differentiated from all the Rocky Mtn. populations in having only a trace of camphene, no limonene, a large amount (53.3%) of  $\beta$ -phellandrene, no borneol, a large amount of piperitone (9.2%), methyl citronellate (0.4%), only a trace of bornyl acetate (0.1%), and a small amount of thymol (2.3%). The oil of *A. l.* var. *bifolia* has considerable amounts of (cpd., MT%, UT%):  $\alpha$ -pinene (8.5, 3.4%), camphene (8.4, 15.0%),  $\beta$ -pinene (10.1, 16.5%),  $\beta$ -phellandrene (6.0, 4.1%), bornyl acetate (21.2, 24.9%) and thymol (3.5, 12.5%). The oil of *A. l.* var. *arizonica* has considerable amounts of  $\alpha$ -pinene (9.2%), camphene (15.2%),  $\beta$ -pinene (24.0%),  $\beta$ -phellandrene (5.1%) and bornyl acetate (34.4%). The oil is differentiated by having no  $\delta$ -3-carene, (E)- $\beta$ -ocimene, trans-p-menth-2-en-1-ol, methyl citronellate, thymol, geranyl acetate or (E)- $\alpha$ -bisabolol. DNA sequencing of nrDNA, trnS-trnG, trnL-trnF, petN-psbM, and psbM-trnD yielded 5655 bp of data. NJ tree and SNPs analyses revealed the corkbark fir (*A. l.* var. *arizonica*) of the southern Rocky Mtns to be the most distinct of the taxa. Based on the oil and DNA data, there is support for the

recognition of *Abies lasiocarpa* var. *lasiocarpa* in the central Rocky Mountains and coastal North America and *Abies lasiocarpa* var. *arizonica* in the southern Rocky Mountains, but little support for the recognition of *A. l.* var. *bifolia*. *Phytologia* 93(1):73-87 (April 1, 2011).

**KEY WORDS:** *Abies lasiocarpa*, var. *bifolia*, var. *arizonica*, Alpine fir, leaf essential oils, DNA sequencing, SNPs, nrDNA, trnS-trnG, trnL-trnF, petN-psbM, psbM-trnD, taxonomy.

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The taxonomy of the infraspecific taxa of *Abies lasiocarpa* (Hooker) Nuttall has been in a state of flux. Recently, Eckenwalder (2009) treated *A. lasiocarpa* as having three varieties: var. *lasiocarpa*, var. *bifolia* (A. Murray bis) Eckenwalder, and var. *arizonica* (Merriam) Lemmon. This is in deference to Hunt (1993) who recognized *A. lasiocarpa* in the coastal region and Cascades and northwesterly into coastal British Columbia and *A. bifolia*, inland in the Rocky Mtns. Hunt (1993) did not recognize var. *arizonica* (corkbark fir), but wrote that "the taxonomy of the corkbark fir ... is uncertain."

Zavarin et al. (1970) examined the monoterpenes from over 400 trees of *A. lasiocarpa* from throughout its range. They found the coastal populations to be high in  $\beta$ -phellandrene and low in limonene, with the inland, Rocky Mtn. populations high in limonene and low in  $\beta$ -phellandrene. The populations in the central and southern Rocky Mtns. appeared to have a different monoterpene pattern (Zavarin et al., 1970, Fig. 8). Of course, that pattern was based on the concentrations of only 3 key compounds:  $\beta$ -pinene, limonene and  $\beta$ -phellandrene.

Hunt and von Rudloff (1979) utilized the volatile leaf oils of *Abies* but published the composition of only the monoterpenes (Table 2, Hunt and von Rudloff, 1979). They found the leaf oils of the coastal populations to be high in  $\beta$ -phellandrene, low in limonene, with only trace amounts of camphene and bornyl acetate. In contrast, they found the Rocky Mtn. populations lower in  $\beta$ -phellandrene, higher in limonene, and with large amounts of camphene and bornyl acetate. Hunt and von Rudloff (1979) concluded that true *A. lasiocarpa* grew only in the northwest coastal mountains and the Rocky Mtn. alpine fir



was *A. bifolia*. This was reflected in Hunt's treatment of *Abies* in Flora of North America (Hunt, 1993).

The present study presents the first complete leaf essential oil (monoterpenes, sesquiterpenes, diterpenes) analyses of 'pure' *A. lasiocarpa* var. *lasiocarpa* (cf. popn. 54, Olympic Peninsula, WA, Hunt and von Rudloff, 1979), *A. l.* var. *bifolia* (cf. popn. 28, Glacier Park, MT, Hunt and von Rudloff, 1979), and *A. l.* var. *bifolia* from Utah as well as *A. l.* var. *arizonica*, Flagstaff, AZ.

Xiang et al. (2009) recently published a phylogeny of *Abies* based on nrDNA sequences. They found *A. lasiocarpa* in a clade with *A. balsamea* and *A. fraseri* sister to a clade of *A. koreana*, *A. veitchii* and *A. nephrolepis*. Other North American species such as *A. amabilis*, *A. concolor*, *A. grandis*, *A. magnifica* and *A. procera* were in different clades (Xiang et al., 2009). However, it is unlikely that nrDNA data alone is sufficient to portray phylogenetic relationships. In this study, we present sequence data for nrDNA, petN-psbM, psbM-trnD, trnL-trnF and trnS-trnD.

## MATERIALS AND METHODS

Leaf samples collected: *A. lasiocarpa* var. *lasiocarpa*: Chris Earle-Adams 12315-12317, Deer Park, Olympic National Park, Olympic Peninsula. 47.948826 N, 123.259027° W, 1643m, June 26, 2010, Clallam Co., WA;

*A. lasiocarpa* var. *bifolia*: Adams 12400-12404, Brighton Ski lodge parking lot. 40° 35' 48.76" N; 111° 35' 09.18" W, 2682m, Sept. 4, 2010, Salt Lake Co., UT; Chris Earle, Adams 12413-12415, 4 mi SW of St. Mary Lodge on US 89. Lat. 48.70142° N, 113.40362° W, 1730 m, Sept. 11, 2010, Glacier Co., MT.

*A. lasiocarpa* var. *arizonica*, Thornburg-Adams 12388-12392, 11 mi. NNW of Flagstaff, at Snowbowl Ski lodge parking lot, 35.32998° N; 111.71194° W, 2826m, Aug. 10, 2010, Coconino Co., AZ. All specimens are deposited in the BAYLU herbarium.

*Isolation of Oils* - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the

samples stored at  $-20^{\circ}\text{C}$  until analyzed. The extracted leaves were oven dried ( $100^{\circ}\text{C}$ , 48 h) for determination of oil yields.

**Chemical Analyses** - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007, for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

**DNA Analysis** - One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, then stored at  $-20^{\circ}\text{C}$  until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia, CA).

The nrDNA region of *Abies lasiocarpa* proved to be too large ( $\sim 1740$  bp) to sequence by use of ITS A and ITS B (Fig. 1).

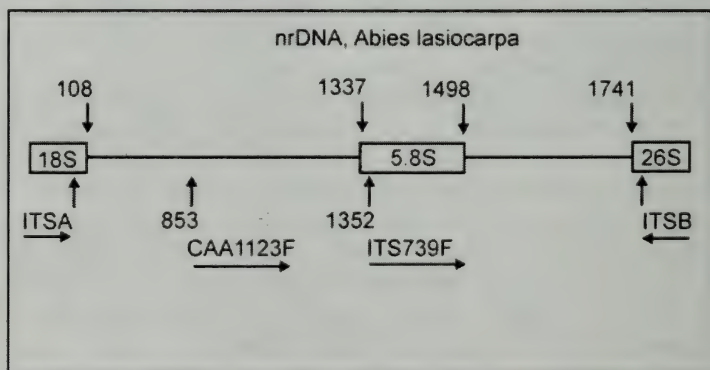


Figure 1. Diagram of the nrDNA region for *Abies lasiocarpa*.

Two addition primers were utilized:  
CAA1123F: AC CTC CTA TGT CGG TTG TGC (Xiang et al. 2009)  
ITS739F: AAC GGA TAT CTC GGC TCT, based on conserved sequences in the 5.8S region.

The trnC-trnD region of *Abies concolor* also proved to be large (~2400, Fig. 2). Due to the small area from trnC to petN, that region was

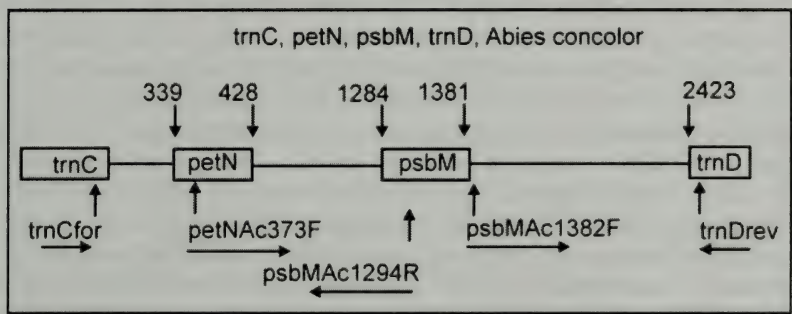


Figure 2. Diagram of the trnC-trnD region of *Abies concolor*.

skipped. Two regions were sequenced: petN-psbM and psbM-trnD using four primers (Fig. 2) based on sequences of *Abies* from GenBank:  
petNAc373F: TGG TAG TTT TTA CAT TTT CC,  
psbMAc1294R: TTA TCC CTT ACG TCA AAA CG  
and  
psbMAc1382F: AGA TCC ATG AAA TAG ATG TG  
trnDrev: GGG ATT GTA GTT CAA TTG GT

Primers for trnL-trnF and trnS-trnG have been previously reported (Adams and Kauffmann, 2010).

PCR amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (cpDNA regions) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl<sub>2</sub> according to the buffer used) 1.8 µM each primer. The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen

QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/>).

Data Analysis - Terpenoids (as percent total oil) were coded and compared among the species by the Gower (1971) metric. Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). DNA data - Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

## RESULTS AND DISCUSSION

In general, the leaf oils of *A. lasiocarpa* are dominated by monoterpenes with only small amounts of sesquiterpenes and diterpenes (Table 1). Table 1 shows the composition of the leaf essential oils of the four taxa. The monoterpenes are in good agreement with Zavarin et al. (1970) and Hunt and von Rudloff (1979). The coastal alpine fir (*A. l.* var. *lasiocarpa*) is very strongly differentiated from all the Rocky Mtn. populations in having only a trace of camphene, no limonene, a large amount (53.3%) of  $\beta$ -phellandrene, no borneol, a large amount of piperitone (9.2%), methyl citronellate (0.4%), only a trace of bornyl acetate (0.1%), a small amount of thymol (2.3%), the presence of  $\alpha$ - and  $\beta$ -selinene, no (E)- $\beta$ -bisabolene, and the presence of 10-epi- $\gamma$ -eudesmol. Interestingly, the lasiocarpenes and juvabiones reported by Manville and Tracey (1989) in the wood of coastal *A. lasiocarpa* were not found in the leaf oils. Apparently, the wood and leaf oils differ in composition (similar to the situation in the Cupressaceae where the leaf and wood oils are quite different).



The oils of *A. l.* var. *bifolia* from Montana and Utah, are similar (Table 1), with considerable amounts of:  $\alpha$ -pinene (8.5, 3.4%), camphene (8.4, 15.0%),  $\beta$ -pinene (10.1, 16.5%),  $\beta$ -phellandrene (6.0, 4.1%), bornyl acetate (21.2, 24.9%) and thymol (3.5, 12.5%). No unique compounds were found in these oils.

The oil of *A. l.* var. *arizonica* from Flagstaff, AZ (Table 1) has considerable amounts of  $\alpha$ -pinene (9.2%), camphene (15.2%),  $\beta$ -pinene (24.0%),  $\beta$ -phellandrene (5.1%) and bornyl acetate (34.4%). The oil is differentiated by having no  $\delta$ -3-carene, (E)- $\beta$ -ocimene, trans-p-menth-2-en-1-ol, methyl citronellate, thymol, geranyl acetate or (E)- $\alpha$ -bisabolol. It contains only a trace of piperitone that is common in the other taxa (Table 1).

To visualize the overall similarities of the oils, principal coordinates ordination (PCO) was performed on a matrix of similarities based on 30 terpenoids (see Table 1). Three eigenroots were extracted and, of course, accounted for all the variation among the four populations. Ordination shows (Fig. 3) that each of the four populations are quite distinct with the oils of putative var. *bifolia* (MT and UT) being the most similar (0.644), followed by var. *arizonica* linking with UT (0.563), and lastly, the coastal, var. *lasiocarpa* population linking with UT (0.518).

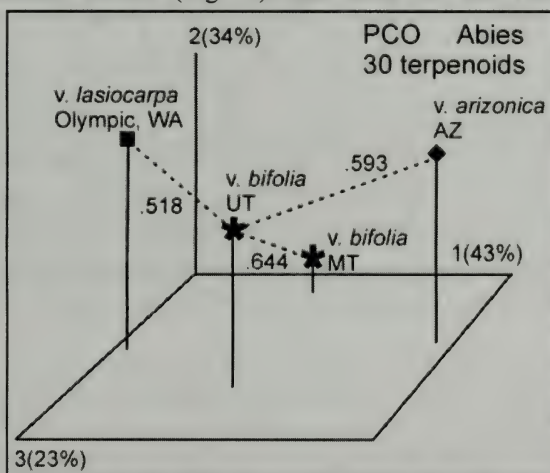


Figure 3. PCO based on 30 terpenoids. The dashed lines are the minimum spanning network (similarities).

Mapping the minimum spanning network (Fig. 4) enables one to obtain a geographic perspective. Clearly the differentiation between coastal alpine fir and Rocky Mtn. fir (cf. Hunt and von Rudloff, 1979) is well supported. It is interesting to note that *A. lasiocarpa*, Olympic Peninsula, is a bit more similar to var. *bifolia* from Utah (0.518) than to Montana (0.484). The linkage of var. *arizonica* with Utah reconfirms the trend found by Zavarin et al. (1970).

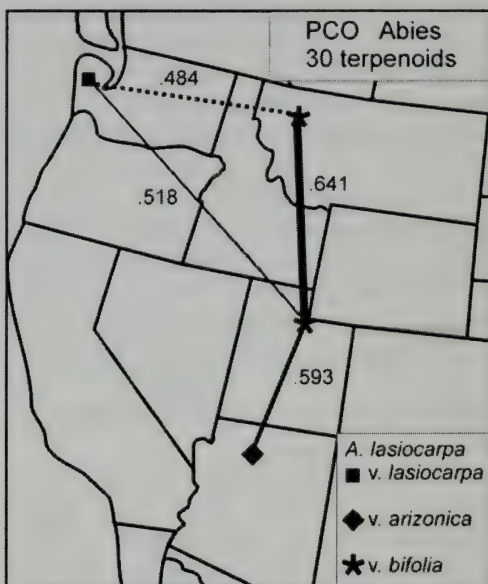


Figure 4. Minimum spanning network based on 30 terpenoids. The dotted line shows the second nearest link for WA (to MT).

Analyses of the concatenated set of nrDNA, trnS-trnG, trnL-trnF, petN-psbM and psbM-trnD sequences resulted in 5655 bp of data. A NJ analysis (Fig. 5) shows each of the four populations of *A. lasiocarpa* in well supported clades. The clade of *A. lasiocarpa* var. *arizonica* is quite distinct (Fig. 5).

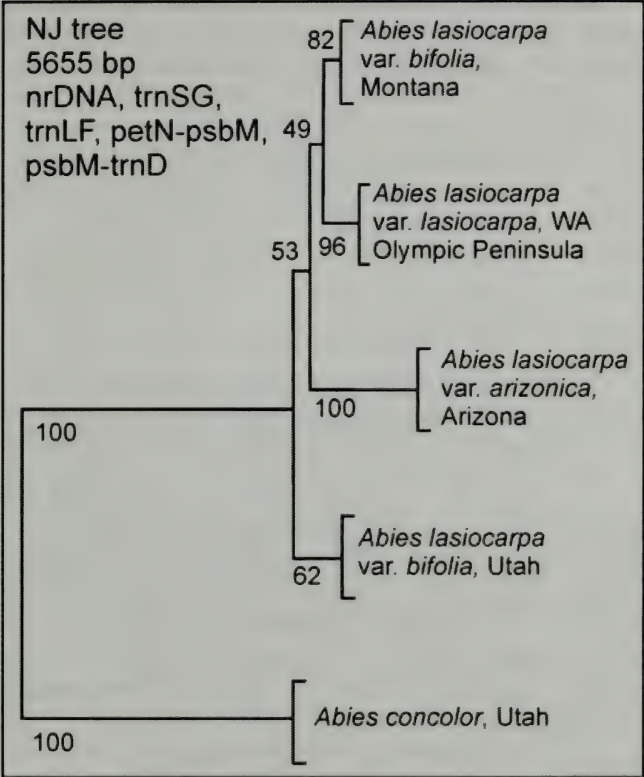


Figure 5. NJ tree based of nrDNA (nuclear) plus cpDNA (trnS-trnG, trnL-trnF, petN-psbM, psbM-trnD). Numbers at the branch points are bootstrap values as percents.

Table 2 shows a summary of the variation in nrDNA, trnS-trnG, trnL-trnF, petN-psbM, and psbM-trnD sequences. Of the large number (135) of substitutions found in nrDNA, 125 were found only in *A. concolor* and this appears to be due to poor alignment as the nrDNA sequence in *A. concolor* contained 11 indels and appeared to be rearranged, making it very difficult to align with *A. lasiocarpa*.

Table 2. Summary of variation found in nrDNA, trnS-trnG, trnL-trnF, petN-psbM, and psbM-trnD sequences. S = population useful base substitution, i = indel, u = unique substitution, found in only one sample.

DNA	<i>A. concolor</i> + <i>lasiocarpa</i>	<i>A. lasiocarpa</i> only
nrDNA	2047bp, 135S, 11i, 0u	1821bp, 10S, 0i, 1u
trnS-trnG	892bp, 10S, 2i, 2u	892bp, 0S, 0i, 2u
trnL-trnF	958bp, 6S, 2i, 0u	958bp, 0S, 1i, 0u
petN-psbM	870bp, 13S, 2i, 0u	868bp, 1S, 0i, 2u
psbM-trnD	927bp, 16S, 5i, 0u	887bp, 2S, 3i, 0u

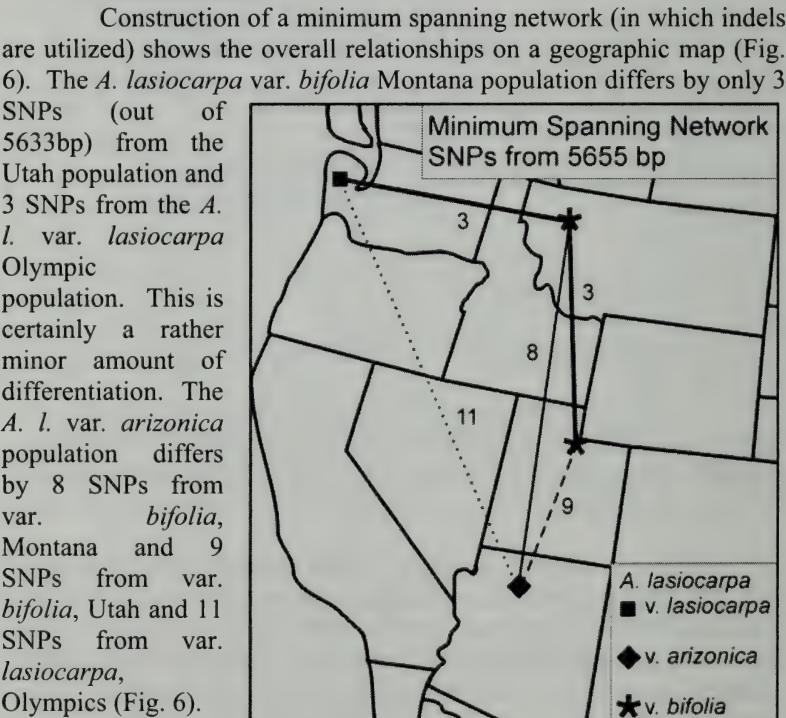


Figure 6. Minimum spanning network based on 5655 bp of sequence data. The numbers next to the links are the number of SNPs (including indel information).



## CONCLUSIONS

The leaf essential oil compositions from these four populations are remarkably differentiated and support the recognition of *A. lasiocarpa* var. *lasiocarpa* from the coastal region of the northwestern US and Canada, and *Abies l.* var. *bifolia* from the northern Rocky Mtns. as suggested by Hunt and von Rudloff (1979) and Manville and Tracey (1989). In addition, the corkbark fir (*A. l.* var. *arizonica*) of the southern Rocky Mtns. is very distinct in its leaf oil and its recognition is supported by these data.

The DNA sequence data is congruent with the oil data of corkbark fir (*A. lasiocarpa* var. *arizonica*) showing it is quite distinct. However, the DNA data gives less support (than the oil data) for the recognition of *A. l.* var. *bifolia* as being distinct from *A. l.* var. *lasiocarpa*.

The conflict between essential oils data and DNA sequence information is not new in conifers. Adams et al. (2005) found perfect taxonomic concordance between nrDNA, RAPDs, leaf essential oils and morphology separating *Juniperus deltoides* R. P. Adams and *J. oxycedrus*. In contrast, Adams et al. (2008) found considerable differences in the classifications of *Juniperus excelsa* M.-Bieb. and *J. polycarpus* K. Koch. varieties between nrDNA, trnC-trnD SNPs and the leaf essential oil data sets. In general, Adams (2008) notes that the leaf essential oils are most useful below the specific level in the study of geographic variation. It seems likely that the alpine fir in the Pacific northwest region (*A. lasiocarpa* var. *lasiocarpa* in this study) is subjected to a quite different set of selection pressures in regards to diseases, insects and herbivores than faced by alpine fir in the Rocky Mountains. Thus, the leaf oils are likely revealing future speciation processes that are not yet apparent in the 'neutral' mutations in the gene introns utilized in this study.

## ACKNOWLEDGEMENTS

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Table 1. Comparison of leaf oil compositions of *Abies lasiocarpa*. Olym = *A. lasiocarpa* v. *lasiocarpa*, Olympic Peninsula, WA, Mont = *A. l.* v. *bifolia*, Montana, Utah = *A. l.* v. *bifolia*, Utah, Ariz = *Abies l.* v. *arizonica*, Arizona. Compounds in bold face appear to separate the taxa. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. RI is the Kovat's Index using a linear approximation on DB-5 column. \*= cpds used for PCO (30 cpds.)

RI	compound	Olym	Mont	Utah	Ariz
884	santene*	t	1.2	1.0	0.4
921	tricyclene*	t	0.7	1.1	0.9
924	$\alpha$ -thujene	t	-	-	-
<b>932</b>	<b><math>\alpha</math>-pinene*</b>	<b>4.2</b>	<b>8.5</b>	<b>3.4</b>	<b>9.2</b>
<b>946</b>	<b>camphene*</b>	<b>0.1</b>	<b>8.4</b>	<b>15.0</b>	<b>15.2</b>
969	sabinene	t	0.6	t	0.4
974	$\beta$ -pinene*	16.6	10.1	16.5	24.0
988	myrcene*	1.6	0.9	0.6	0.7
1002	$\alpha$ -phellandrene*	0.6	0.3	0.3	0.1
<b>1008</b>	<b><math>\delta</math>-3-carene*</b>	<b>0.7</b>	<b>4.1</b>	<b>0.3</b>	-
1014	$\alpha$ -terpinene	0.2	0.2	0.2	t
1020	p-cymene	t	t	t	t
<b>1024</b>	<b>limonene*</b>	-	<b>17.6</b>	<b>8.4</b>	<b>2.5</b>
<b>1025</b>	<b><math>\beta</math>-phellandrene*</b>	<b>53.3</b>	<b>6.0</b>	<b>4.1</b>	<b>5.1</b>
1038	2-heptyl acetate	-	t	0.1	-
<b>1044</b>	<b>(E)-<math>\beta</math>-ocimene*</b>	<b>0.5</b>	<b>0.3</b>	<b>0.1</b>	-
1054	$\gamma$ -terpinene	0.2	0.2	0.1	0.1
1065	cis-sabinene hydrate	t	t	t	t
1086	terpinolene*	1.0	1.3	0.8	0.4
1095	6-camphenone	-	-	-	t
1095	linalool	0.2	0.4	0.3	0.2

RI	compound	Olym	Mont	Utah	Ariz
1098	trans-sabinene hydrate	-	t	-	-
1118	endo-fenchol	-	-	-	t
<b>1118</b>	<b>cis-p-menth-2-en-1-ol*</b>	<b>0.9</b>	<b>0.5</b>	<b>1.0</b>	<b>t</b>
1122	$\alpha$ -campholenal	t	-	t	t
1135	trans-pinocarveol	-	-	-	t
<b>1136</b>	<b>trans-p-menth-2-en-1-ol*</b>	<b>0.8</b>	<b>0.5</b>	<b>0.8</b>	-
1141	camphor*	0.1	0.3	0.4	0.7
1145	camphene hydrate	-	0.2	0.2	0.1
1148	citronellal	-	t	0.1	t
1160	pinocarvone	t	-	-	-
1165	p-mentha-1,5-dien-8-ol	-	0.1	t	-
<b>1165</b>	<b>borneol*</b>	-	<b>0.4</b>	<b>0.4</b>	<b>2.9</b>
1172	cis-pinocamphone	-	-	-	0.1
1174	terpinen-4-ol*	0.4	0.6	0.3	0.3
1186	$\alpha$ -terpineol*	0.3	0.5	0.5	0.3
1195	cis-piperitol	0.2	0.1	0.2	-
1195	myrtenal	-	-	-	t
1195	myrtenol	0.2	0.1	0.3	t
1204	verbenone	-	-	-	t
1207	trans-piperitol*	0.4	0.3	0.4	-
1218	endo-fenchyl acetate	-	t	0.1	-
1223	citronellol*	t	0.4	t	0.4
1232	thymol, methyl ether*	1.0	0.6	t	t
<b>1249</b>	<b>piperitone*</b>	<b>9.2</b>	<b>4.9</b>	<b>1.4</b>	<b>t</b>
<b>1257</b>	<b>methyl citronellate*</b>	<b>0.4</b>	<b>t</b>	<b>t</b>	-
1274	neo-isopulegol acetate	-	-	t	t
<b>1287</b>	<b>bornyl acetate*</b>	<b>0.1</b>	<b>21.2</b>	<b>24.9</b>	<b>34.4</b>
<b>1289</b>	<b>thymol*</b>	<b>2.3</b>	<b>3.5</b>	<b>12.5</b>	-
1298	trans-pinocarvyl acetate	-	-	-	t
132	cis-piperityl acetate	t	-	-	-
1350	citronellyl acetate	t	t	0.1	-
1359	neryl acetate	t	-	-	-
<b>1379</b>	<b>geranyl acetate*</b>	<b>1.1</b>	<b>0.1</b>	<b>0.4</b>	-
1387	$\beta$ -bourbonene	-	t	-	-
1389	longifolene	-	t	0.2	-
1400	$\beta$ -longipinene	-	-	-	t



RI	compound	Olym	Mont	Utah	Ariz
1436	isobazzanene	-	0.1	-	-
1458	allo-aromadendrene	t	-	-	-
1480	allo-aromadendr-9-ene	-	0.1	t	-
<b>1489</b>	<b><math>\beta</math>-selinene</b>	<b>0.1</b>	<b>t</b>	<b>t</b>	<b>t</b>
1493	$\delta$ -decalactone	-	0.2	t	t
<b>1498</b>	<b><math>\alpha</math>-selinene</b>	<b>0.1</b>	-	-	-
1500	$\alpha$ -muurolene	-	t	-	-
1505	$\beta$ -bisabolene	0.1	t	t	-
<b>1539</b>	<b>(E)-<math>\beta</math>-bisabolene*</b>	-	<b>0.7</b>	<b>0.2</b>	<b>0.1</b>
1561	(E)-nerolidol	0.1	-	0.1	t
<b>1622</b>	<b>10-epi-<math>\gamma</math>-eudesmol</b>	<b>0.2</b>	-	-	-
1656	valerianol	0.3	t	t	-
1658	neo-isointermedeol	-	t	t	-
<b>1683</b>	<b>(E)-<math>\alpha</math>-bisabolol*</b>	<b>0.5</b>	<b>0.3</b>	<b>0.5</b>	-
1981	isopimara-8(14),15-diene	t	t	t	-
1987	manoyl oxide	t	0.1	t	t
2014	palustradiene(=abieta-8, 13-diene) *	0.2	0.7	0.5	0.1
2055	abietatriene*	0.2	0.4	0.7	0.1
2087	abietadiene	t	0.1	0.1	t
2149	abienol	0.2	t	t	t
2300	tricosane(C23)	-	t	t	0.1
2313	abietal	-	0.1	t	t

A NEW GYPSOPHILIC *PHACELIA* (HYDROPHYLLACEAE)  
FROM COAHUILA, MEXICO

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ABSTRACT

A novel *Phacelia*, *P. marshall-johnstonii* var. *deliciasana* B.L. Turner, **var. nov.**, is described from south-central Coahuila where it occurs in gypsum soils. It is closely related to *P. gypsogenia* and *P. marshall-johnstonii*, but less so to the former. It is readily distinguished from the latter by its strongly perennial habit, less markedly pubescent leaves, and more elongate fruiting calyces. Relationships of the several phacelioid gypsophiles of Coahuila are discussed and a key to the taxa is provided, along with maps showing their distributions. *Phytologia* 93(1):88-93 (April 1, 2011).

**KEY WORDS:** *Phacelia*, Hydrophyllaceae, gypsophiles, Mexico, Coahuila

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Routine identification of Mexican plants has occasioned the following novelty:

**PHACELIA MARSHALL-JOHNSTONII** var. **DELICIASANA**  
B.L. Turner, **var. nov.**, **Fig. 1.**

*Phacelia marshall-johnstonii* I.M. Johnst. similis sed differt duratione perenni, habitu radice palari, foliis strigosis trichomatibus plerumque sparsis appressis brevibus (vs dense pubescentibus trichomatibus rigide erectis elongatis), et calycibus fructiferis lineari-oblongatis 5-7 mm longis (vs magis paene obovatis, 4-5 mm longis).

**TYPE: MEXICO. COAHUILA: Mpio. San Pedro de Los Colonias,** ca 2 mi. SW of Las Delicias, gypsum soils, ca 26 12 N, 102 49 W, 1150 m, 10 Jun 2004, *Henrickson 23581* (Holotype: TEX).

**Tap-rooted, perennial herbs,** 10-30 cm high. **Mid-stems** densely pubescent with mostly eglandular hairs ca 0.5 mm high, interspersed among these a lesser display of stiffly erect, eglandular, hairs ca 2 mm long. **Leaves** (mid-stem), 3-7 cm long, 2-3 cm wide; petioles 1.5-3.0 cm long, pubescent like the stems; blades elliptical to flabellate, pubescent above and below with appressed, eglandular, hairs, the margins irregularly incised with shallow lobes. **Capitulescence** a terminal array of 2-10 circinnate racemes 3-7 cm long (to 15 cm long in fruit), pubescent like the stems. **Calyces** (flowering) having 5 separate lobes, 3-4 mm long, pubescent like the stems, elongating in fruit to 6-7 mm long, and becoming markedly oblanceolate. **Corollas** white, glabrous, ca 6 mm long, the tubes ca 1 mm long, the throat, including lobes, ca 5 mm long. **Anthers** purple. **Style branches** fused for ca 3 mm at the base. **Capsules** ovoid, ca 2 mm long, 2 mm wide, glabrous below, pubescent above. **Seeds** black, 2.0-2.5 mm long. **Distribution** see Map 1.

**ADDITIONAL SPECIMEN EXAMINED: MEXICO. COAHUILA: Mpio Gral. Cepeda,** Canyon Carrera, SW quadrant of Sierra de la Paila, 1450-1750 m, 26 Jul 1993, *Patterson et al. 7259* (TEX). **Mpio San Pedro de las Colonias,** 12 km NNE of Las Margaritas on the easternmost ridge of Sierra de las Margaritas, 1300-1400 m, 24 Sep 1972, *Chiang et al. 9509B* (LL); ca 1.5 mi SW of Las Delicias, W of the major spring above town, 3900 ft, 15 Aug 1973, *Henrickson 12457* (LL); 1.5 mi SSE and above Las Delicias, S of major spring, 3700 ft, 29 Sep 1973, *Henrickson 13685* (TEX); west end of the Sierra de los Alamitos, 3 miles S of El Mesquite, on and below distinct w-facing gypsum slopes visible from Hwy 30, 26 20 68 N, 102 37 5 W, 3000 ft, 1 Sep 2004, *Henrickson 24021* (TEX); E side of Sierra de Las Margaritas, ca 13 km N of Las Margaritas, 1100-1400 m, 23 Mar 1973, *Johnston et al. 10353* (LL). **Mpio. Parras de la Fuente,** Parras, S slope of Sierra de Parras, 1945 m, 11 Sep 1999, *Hinton et al. 27465* (TEX);

The var. *deliciasana* is clearly related to the typical elements of *P. marshall-johnstonii*, to which it is compared in the above diagnosis, the latter possessing a markedly spreading, densely setose-

like pubescence on both stems and leaves, this not found in the former. I was inclined to treat var. *deliciasana* at the specific level when first discerned, but collections from the Parras area of southern Coahuila (e.g., *Patterson 7259*) showed a tendency to grade into the former, hence its treatment as a variety. The distributions of the five taxa concerned are shown in Fig. 2.

The specific name is derived from the Sierra Las Delicias, whence the type.

The several gypsophiles of *Phacelia* in north-central Mexico are all closely related and occur in close proximity to one another, but each is readily recognized by a combination of characters, and so far as known, they do not occur together, although occasional co-occurrences are to be expected in regions of nearness.

The original member of the pentad of gypsophiles discussed here, *P. gypsogenia* I.M. Johnst. was first collected, and subsequently described, by Johnston (1941), this, for some reason, not accounted for in Atwood's seminal treatment of the *Phacelia* Crenulatae group of North America. Johnston (1943) also added the second taxon to the group with his description of *P. pallida*. Atwood (1972) added a third species to the complex, *P. vossii*; originally known only by the type, but subsequently numerous collections have been assembled (LL, TEX). A fourth member of this pentad, *A. marshall-johnstonii*, was proposed by Atwood & Pinkava in 1977 from material collected in the vicinity of Cuatro Cienagas, Coahuila; a fifth member of the pentad, *P. m.* var. *deliciasana*, from south-central Coahuila is described above. The following key should prove helpful in their identification:

1. Leaves, at mid-stem with well-defined petioles 1.0-1.5 cm long the blades mostly weakly incised, elliptical to flabellate in outline, 1.5-4.5 cm long, mostly pubescent on both surfaces with short appressed hairs; sepals in fruit 6-7 mm long; corollas mostly white; vicinity of Las Delicias.....***P. m.* var. *deliciasana***
1. Leaves not as described in the above; sepals in fruit mostly 3-5 mm long; corollas mostly pale lavender to purple (rarely white).....(2)



2. Robust perennial herbs 30-80 cm high; corollas mostly deep purple; seeds ca 3.5 mm long Nue, San and Zac.....**P. vossii**
2. Smaller, annual (?) or perennial herbs 10-30 cm tall; corollas pale purple to white; seeds 2.0-2.5 mm long; Chi, Coa, Nue, Dur and Zac.....**(3)**
3. Capsules ellipsoid, 3.0-4.0 mm long; seeds ca 3 mm long; Brewster Co., Tx and closely adjacent Mexico.....**P. pallida**
3. Capsules globoid, 2.5-3.0 mm long; seeds 2.0-2.5 mm long; widespread in northern Mexico.....**(4)**
4. Leaves linear-lanceolate in outline, the blades markedly incised, 6-9 cm long; Chi, Coa, Nue, Dur and Zac.....**P. gypsogenia**
4. Leaves elliptical to flabellate in outline, not markedly incised, mostly 3-5 cm long; vicinity of Cuatro Cienagas, Coa.....  
.....**P. m. var. marshall-johnstonii**

## ACKNOWLEDGEMENTS

My colleague, Guy Nesom, provided the Latin diagnosis and made helpful suggests on the manuscript itself. Distribution maps are based upon specimens on file at SRSC, LL-TEX, and those cited by various authors.

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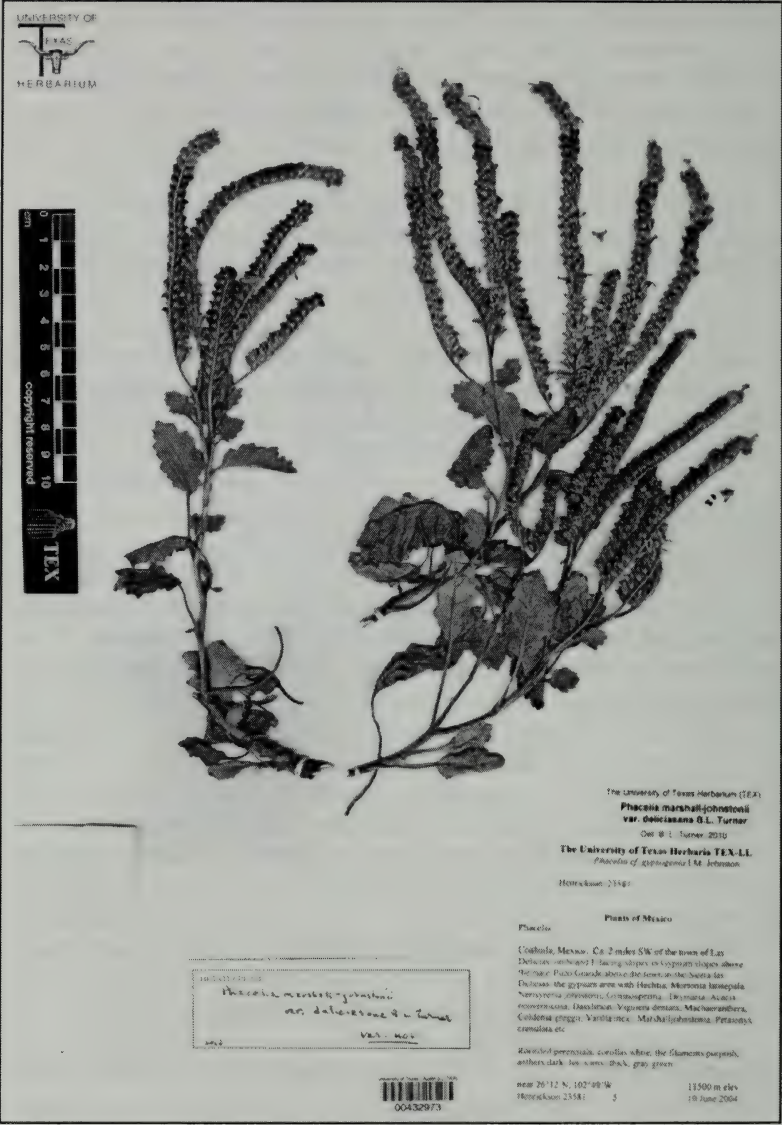
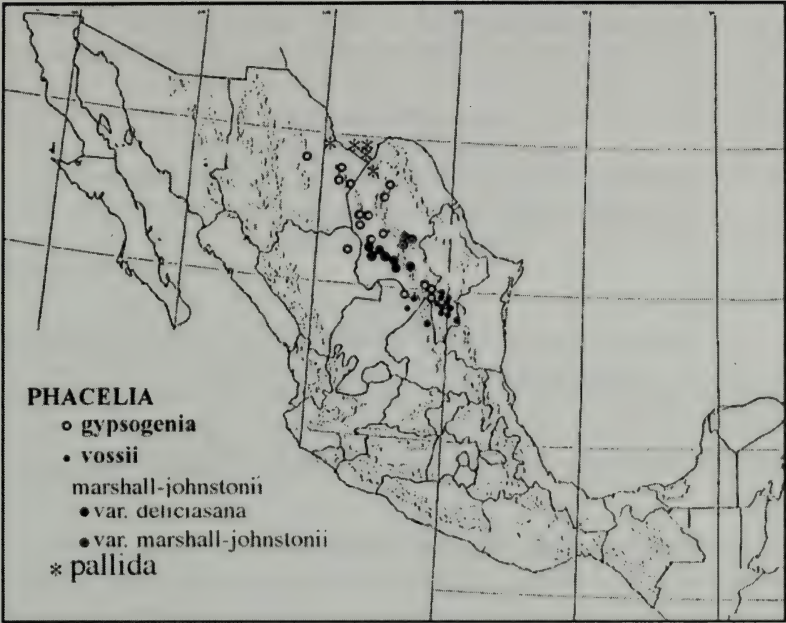


Figure 1. Holotype of *Phacelia marshall-johnstonii* var. *deliciosana*.



Map 1. Distribution of *Phacelia* species in Mexico.

*AGERATINA TOVARAE*, A NEW SPECIES FROM  
NORTHERN PERU (ASTERACEAE: EUPATORIEAE)

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**ABSTRACT**

Based on examination of specimens, a new species, *Ageratina tovarae* is recognized from Peru. *Phytologia* 93(1): 94-98 (April 1, 2011).

**KEY WORDS:** Key words: *Ageratina tovarae*, Cajamarca, Peru, new species

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Over 270 species of *Ageratina* Spach are recognized in North America, Central America and western South America. These include the species noted when the genus was first resurrected (King & Robinson 1970) and many added since (King & Robinson 1972, 1987; Robinson 1990, 2001, 2003, 2008; Turner 1997, 2008, 2009).

At this time an additional new species of *Ageratina* needs to be described from northern Peru. Material of the new species has been seen since 1983 and 1984, collected by D.N. Smith, and it has been variously identified as two other Andean species, *Ageratina scopulorum* (Wedd.) R.M. King & H. Rob. or *A. gracilis* (H.B.K.) R.M. King & H. Rob. Recent collections from an ecological study by Carolina Tovar have resulted in a more thorough review of the entity. A position in the typical subgenus *Ageratina* is confirmed, but the species is distinct from either of the two species with which it has previously been identified. The characteristic that all three species share is the comparatively small size, but the species described here is easily distinguished by the clustering of stems at the base of the plant and the short white mostly appressed to slightly spreading puberulence. *Ageratina gracilis*, a primarily Colombian species, is similar to the new



species in the very short petioles, 1-2 mm long, but it is a more commonly decumbent plant with less crowded stems and reddish hairs. *Ageratina scopulorum*, mostly from southern Peru, has less erect stems, petioles usually 3-5 mm long, and more triangular leaves with truncate bases on the blades. The latter species also has more numerous short outer involucre bracts with dark tips. None of the other species of *Ageratina* seen from the area of the northern Andes shows the consistently short white pubescence on the stems and inflorescence seen in the new species.

The new species is named here for the recent collector Carolina Tovar, niece of the botanist Oscar Tovar.

(*Ageratina tovarae* H. Rob., sp. nov. Type: Peru: Cajamarca. Dist. Cajamarca, -78.5438° W -7.0829° S: Alt. 3368 m: 16 June 2009: C. Tovar B 4599 M 1327 (holotype US; isotype CPUN). (Fig. 1).

In habitu parvo fruticosus basaliter dense aggregatis et in caulibus saepe non ramosis et in puberulescentibus brevibus albis plerumque appressis distincta.

Small weak shrubs with stems closely clustered on basal rhizomes or small xylopodia. Stems slender, woody, not or sparsely branching above base, branches strongly ascending; surfaces often reddish, puberulous with short, white, mostly appressed to slightly spreading hairs that give surfaces of stems, branches, peduncles and sometimes involucre bracts a whitish appearance. Leaves opposite, with short petioles 1-2 mm long; blades small, narrowly ovate to lanceolate, 9-24 mm long, 4-12 mm wide, slightly carinate, bases subacute, margins above broadest part with blunt serrations, appearing nearly entire, apex strictly acute, surfaces without evident glandular dots, with small hairs on main veins and margins of petioles and base of blade, triplinervate with a pair of strongly ascending secondary veins from near base of blade. Inflorescence terminal, weakly cymiform, with strongly ascending branches, with 1-7 heads per branch; reduced foliiform to linear bracteoles borne alternate at few nodes below heads, peduncles slender, 5-20 mm long. Heads broadly campanulate; involucre 5-7 mm wide, 4-6 mm high; bracts eximbricate, ca. 17, with few shorter bracts outside, inner bracts short-

acute, with scarious tips, with few pale hairs on outer surface. Florets ca. 25 in a head; corollas white, ca. 4.5 mm long, basal tube strongly constricted, ca. 1.5 mm long, throat narrowly funnelform, ca. 2.2 mm long, lobes longer than wide, ca. 0.7 mm long, without evident hairs outside; anther thecae ca. 1 mm long; style base distinctly enlarged, glabrous; style branches slightly broader distally. Achenes ca. 2.3 mm long, slightly fusiform, with setulae on ribs and sides, carpodium stopper-shaped; inner pappus series of fragile capillary bristles ca. 4 mm long, broader in distal half, outer pappus series of narrow scales ca. 0.5 mm long.

Paratypes: Peru: Cajamarca. Cajamarca Prov., Cajamarca – Celendín road, between Cajamarca and Encañada, ca. km 25 (78° 24' W, 7°09' S); 3000 m, Dry shrubby grassland in area of intermittant agriculture, heavily overgrazed, limestone; 15 July 1983; *D.N. Smith & I. Sanchez 4238* (MO, US); Cajamarca – Celendín road, between Cajamarca and Pampa de la Culebra; 3000 m, agricultural land with very disturbed patches of dry, low shrubland; 31 May 1984; *D.N. Smith 7367* (MO, US); Dist. Jesus, -78.338° W -7.25° S; Alt. 2985 msnm, s.d.; *Tovar P5647 M054* (CPUN, US); Dist. Los Baños Delinca, Cajamarca, -78.4539° W -7.0629° S; Alt. 3342 msnm; 16 June 2009; *Tovar B 4457 M1342* (CPUN, US). Where stated the plants are consistently referred to as erect shrubs with white flowers.

### ACKNOWLEDGEMENTS

Carolina Tovar is thanked for sending the specimens which were forwarded through Anton Cleef in Amsterdam. Ingrid Pol-yin Lin of the Department of Botany at the U.S. National Herbarium is thanked for the scan of the type specimen. I also thank Guy Nesom, one of the reviews of the manuscript.

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Figure 1. *Ageratina tovarae* H. Rob., holotype, Tovar B4599 M1327 (US).



## RECENSION OF THE GENUS MALACOMELES (ROSACEAE)

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### ABSTRACT

A recension of the mostly Mexican genus *Malacomeles* is rendered. Five species are recognized, two of these, **M. pringlei** (Koehne) B.L. Turner, **comb. nov.** and **M. psilantha** (C. K. Schneider) B.L. Turner, **comb. & stat. nov.**, elevated to specific status from varietal rank. A key to the taxa is provided, along with distribution maps. *Phytologia* 93(1):99-106 (April 1, 2011)

**KEY WORDS:** *Malacomeles*, Rosaceae, Mexico

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### MALACOMELES (Decne.) G.N. Jones

Unarmed shrubs or small trees to 8 m high. Leaves simple, alternate, petiolate, pinnately veined, entire to denticulate, the surfaces glabrous to variously pubescent. Flowers regular, perfect, corymbose to paniculate, bracteate; hypanthium campanulate to urceolate; calyx 5-lobed, persistent, reflexed in fruit; petals 5, white or somewhat rosy, usually wider than long; stamens ca 20, inserted on the calyx rim; styles 3-5, free to the base; ovary inferior, 3-5 loculate, each locule with 2 ovules; fruit a 6-10 loculate pome with 1 seed in each locule; seeds brown, flattened, smooth. **TYPE SPECIES:** *Malacomeles denticulata* (Kunth) G.N. Jones

## Key to species

1. Pedicels glabrous; larger leaf-blades mostly 1.0-1.5 times as long as wide, their apices markedly denticulate, under-surfaces sparsely to moderately puberulent; Chihuahua, Coahuila, Nuevo Leon and closely adjacent Trans-Pecos, Texas.....**M. pringlei**
1. Pedicels to some extent pubescent; larger leaf-blades mostly 1.5-6.0 times as long as wide, their apices only weakly dentate, if at all, the under surfaces mostly densely puberulent; central Mexico to Guatemala.....(2)
2. Larger leaves mostly 1-3 cm long, broadly obtuse and sparsely dentate at apex.....**M. denticulata**
2. Larger leaves mostly 3-10 cm long, scarcely dentate, if at all.....(3)
3. Leaf-blades mostly 6-10 cm long, acute to broadly obtuse at apices, Nuevo Leon and Tamaulipas.....**M. paniculata**
3. Leaf-blades mostly 3-6 cm long, broadly rounded to obtuse at apices.....(4)
4. Calyces and outer receptacles densely pubescent; Chiapas, Guatemala.....**M. nervosa**
4. Calyces and outer receptacles glabrous; north-central Mexico to Oaxaca.....**M. psilantha**

**MALACOMELES DENTICULATA** (Kunth) G.N. Jones, Madrono 8: 36. 1945.

*Cotoneaster denticulata* Kunth, in H.B.K. 1823

*Mespilus denticulata* (Kunth) Spreng. 1825

*Nagelia denticulata* (Kunth) Lindl. 1845

*Amelanchier denticulata* (Kunth) K. Koch 1869

*Crataegus minor* Sesse & Moc. 1887

*Crataegus inermis* Sesse & Moc. 1887

TYPE: MEXICO. HIDALGO: Mpio. Actopan, Actopan, Bonpland s.n.

Nue, Zac, San, Que(?), Hid, Mex, Pue and Oax, gypseous or calcarious soils, 500-1600 m; flowering: Jul-Nov

Shrubs, mostly 1-4 m high; occurring with or near *M. psilantha*, and presumably forming hybrids with the latter upon occasion.

Jones (1945) included *M. pringlei* within the fabric of this species, the latter readily distinguished by a number of characters, as noted in the above key and discussed below.

**MALACOMELES NERVOSA** (Decne.) G.N. Jones, Madrono 8: 38. 1945.

*Cotoneaster nervosa* Decne. 1874

*Amelanchier denticulata* var. *nervosa* (Decne.) C. K. Schneid. 1906

*Nagelia denticulata* var. *nervosa* (Decne.) C. K. Schneid. 1907

TYPE: **MEXICO. CHIAPAS:** "Regno Mexicano, Prov. Chiapa." Feb. 1840, *Linden s.n.*

Cps and Guatemala,, 1300-2000 m; flowering: all seasons

Shrubs or small trees to 6 m high. Much resembling *M. psilantha* but readily distinguished by the above key characters. Occasional plants have markedly denticulate leaves.

**MALACOMELES PANICULATA** (Rehder) J.B. Phipps, Canad. J. Bot. 68: 2234. 1990.

*Amelanchior paniculata* Rehder

TYPE: **MEXICO. Nuevo Leon: Mpio. Galeana**, "ca 15 mi s.w. of Galeana." 9 May 1934, *C.H. & M.T. Mueller* 282.

Nue and Tam, calcareous or gypseous soils, 1500-2500 m; flowering: Nov-Feb.

Large shrubs or small trees, mostly 3-8 m high; resembling *M. psilantha* and occurring with or near the latter upon occasion, but readily distinguished by its larger, less rounded leaves, as noted in the above key. Although the two taxa frequently grow in close proximity, hybrids between these have not been detected

Jones (1945) placed *M. paniculata* within his broad concept of *M. nervosa*, but Phipps accepted its specific status, as do I. According to label data, the former is a small tree occurring at higher elevations and flowering mostly in late fall and early spring. James Henrickson (by annotation, TEX) also accepted its specific status.

/ **MALACOMELES PRINGLEI** (Koehne) B.L. Turner, **comb. nov.**  
Based upon *Amelanchier pringlei* Koehne, Gattun. Pomac., in Wissen. Beil. Progr. Falk.- Real. Berlin 95: 25. 1890.

Chi, Coa and Nue, mostly calcarious soils, 700-1600 m;  
flowering: Mar-Jul.

Shrubs mostly 1-3 m high; resembling *M. denticulata*, but readily distinguished by the characters called to the fore in the above key. In Nue it occurs with or near *M. psilantha* and probably forms hybrids with that taxon upon occasion.

James Henrickson (by annotation, TEX) also recognized the specific status of this taxon, but Jones (1945) treated it within his broad concept of *M. denticulata*.

/ **MALACOMELES PSILANTHA** (C. K. Schneid.) B.L. Turner,  
**comb. & stat. nov.**  
Based upon *Amelanchier denticulata* var. *psilantha* C. K. Schneid., Ill. Handb, Laubh. 1: 743. 1906.  
*Nagelia denticulata* var. *psilantha* (C. K. Schneid.) C. K. Schneid., 1907

TYPE: MEXICO. TAMAULIPAS: **Mpio. Tula**, Tula, Gregg 599.

s Coa, Nue, sTam, s Dur, San, Gua, Que, Ver, Pue and Oax,  
various soils. 700-1500 m; flowering: Aug- Dec.

Shrub 1-4 m high; closely related to *M. denticulata* and *M. nervosa*, but seemingly distinct from both, as noted in the above key.

Jones (1945) failed to recognize *M. psilantha*, placing this within his broad concept of *M. nervosa*. James Henrickson (by annotation, TEX), treated it as a variety of the latter. I think it best treated at the specific level, for it is markedly distinct from the



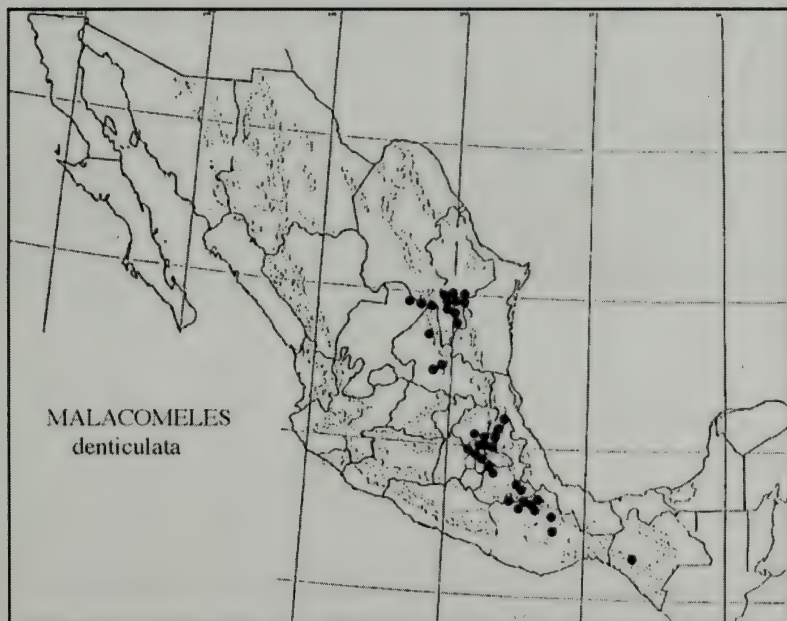
geographically isolated, *M. nervosa*, and possessed of well-marked distinguishing characters.

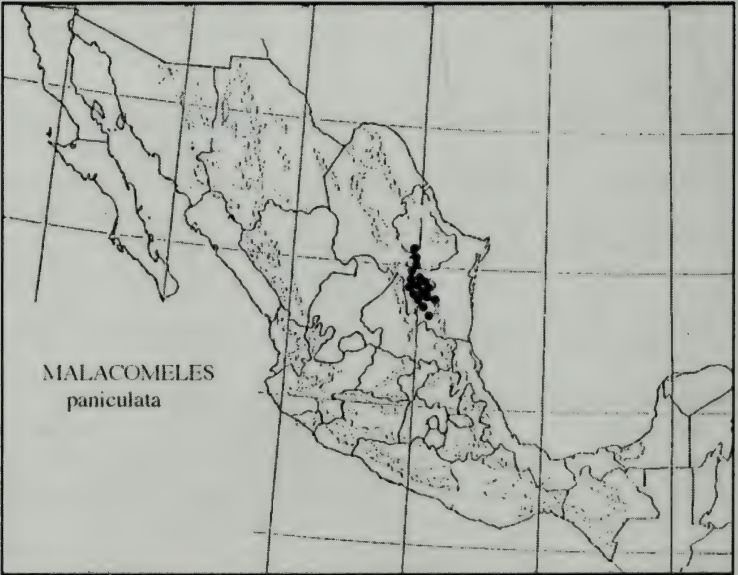
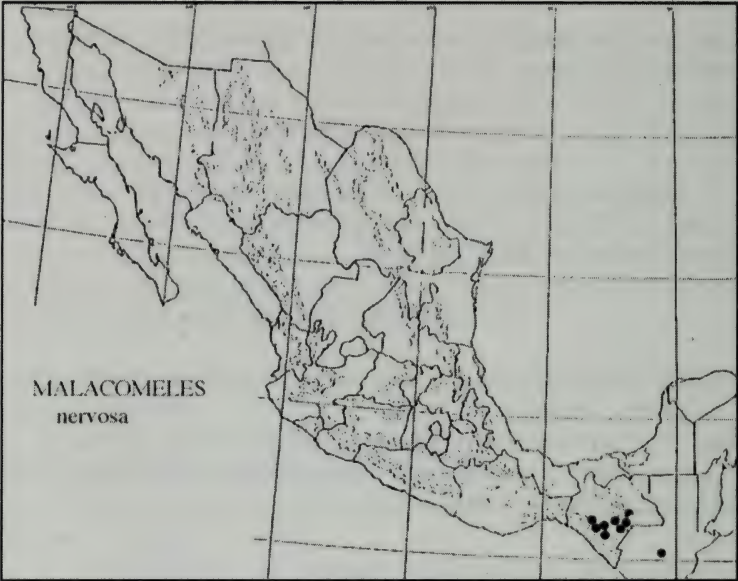
### ACKNOWLEDGEMENTS

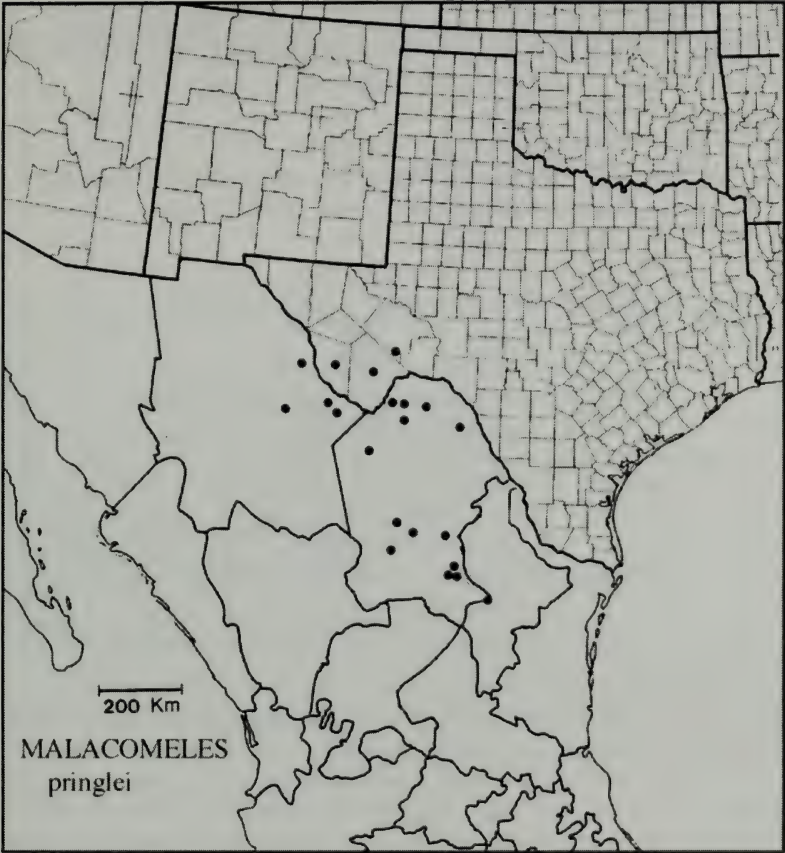
Distribution maps (attached and alphabetically) are based upon specimens on file at LL-TEX. My colleague A. M. Powell provided helpful suggestions on the paper itself.

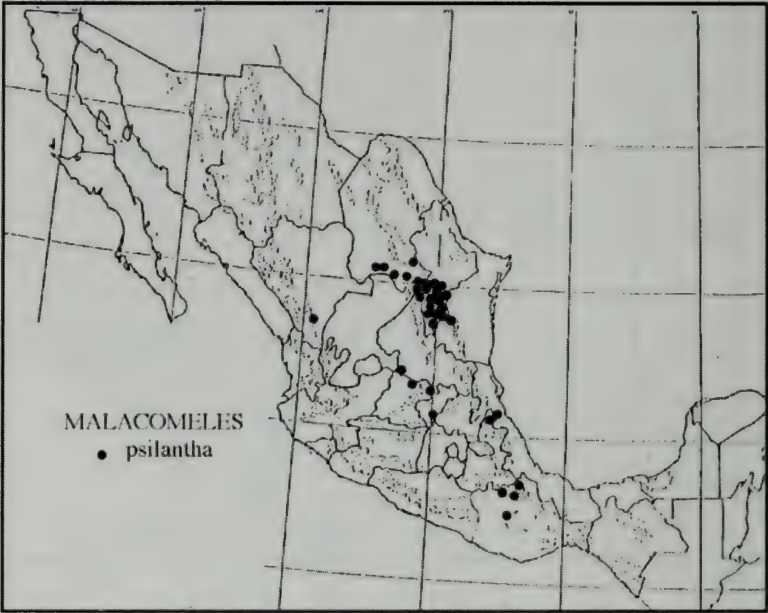
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**TAXONOMY OF INFRASPECIFIC TAXA OF *ABIES*  
*CONCOLOR*: LEAF ESSENTIAL OILS OF *VAR. CONCOLOR*  
AND *VAR. LOWIANA***

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**ABSTRACT**

The leaf essential oils of *Abies concolor* var. *concolor* and var. *lowiana* had large amounts of  $\beta$ -pinene (41-52%), except the Cimarron, NM population had 3.1%  $\beta$ -pinene. The oils from central and northern California were very similar and were devoid of (E)- $\beta$ -ocimene and 6-methyl-5-octen-one. The New Mexico oil was the most unusual being high in camphene (25.9%), bornyl acetate (28.7) and  $\alpha$ -pinene (11.2) with several unique components. Populations of *Abies c.* var. *lowiana* in central and northwest California were uniform in their leaf oil compositions. In var. *concolor*, considerable differentiation was found, confirming the work of Zavarin et al. (1975) of the Cuyamaca Race, and three sub-types of *A. c.* var. *concolor* oils: group A, B1 and B2. The B1 oil (Cimarron, NM population) was very different from any other populations, warranting additional studies. *Phytologia* 93(1): 107-117 (April 1, 2011).

**KEY WORDS:** *Abies concolor* var. *concolor*, *A. c.* var. *lowiana*, leaf essential oils composition, geographic variation.

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*Abies concolor* (Gord. and Glend.) Hilde. is a forest tree of western North America (Fig. 1) ranging from Oregon to northern Mexico (Zavarin et al. 1975). Eckenwalder (2009) recognized two varieties: var. *concolor* and var. *lowiana* (Gord.) Lemm. and noted that these have been treated as species by some authors. He also indicated that var. *lowiana* hybridizes with *A. grandis* (D. Doug. ex D. Don in Lamb.) Lindl. but not with *A. lasiocarpa* (Hook.) Nutt. Recently, Xiang et al. (2009) examined nrDNA sequence data and found *A. concolor* most closely related to *A. grandis*, so hybridization seems possible. Zavarin et al. (1975) analyzed wood monoterpenes of *A. concolor* from 43 populations and found evidence that var. *lowiana* (n. and c. California) was a group (Fig. 1), but called var. *lowiana* from s. California, the Cuyamaca race. In addition, Zavarin et al. (1975) subdivided var. *concolor* into three groups (A, B1 and B2, Fig. 1).

There appears to be only one paper reporting on the leaf essential oil of *A. concolor* (Wagner et al. 1989) from a population in the North Kaibab Ranger District, AZ, and those data were reported on a ppm basis instead of the normal percent total oil data.

The purpose of this research was to examine the leaf essential oils from *A. concolor* from major chemical types found by Zavarin et al. (1975) and report on their compositions.

## MATERIALS AND METHODS

Plant specimens: *Abies concolor* var. *concolor*: Adams 12405-12407, Mill B trailhead, Big Cottonwood Canyon, Salt Lake City, UT, 40° 37.996' N, 111° 43.418' W, 6242 ft., Adams 12481-12485, (by D. Thornburg) 7 mi. nw of Pine, AZ along Rim Rd., 34° 26.844'N, 111° 21.520'W, 7597 ft., Adams 12556-12560, 7 mi. w of Cimarron, NM on US 64, 36° 32.81' N, 105° 02.0' W, 6980 ft.,

*Abies concolor* var. *lowiana*: Adams 12427-12431 (by R. Lanner) 2 mi. n of jct. US50 on White Meadows Rd., ca. 22 mi e of Placerville, CA, 38° 47' 00" N, 120° 29' 20" W, 3450 ft., Adams 12432-12436 (by R. Lanner) Mormon Emigrant Trail at jct. with Park Creek Rd., ca. 24 mi ese of Placerville, CA, 38° 43' 30" N, 120° 28' 20" W, 4000 ft., Adams 12438-12442 (by M. Kauffmann) Klamath Mtns., CA, 40° 50' 21.4" N, 123° 43' 11.09" W, 4820 ft., Adams 12464-12468 (by B. Miller) Lee

Summit, CA on Hwy 70/89, 39° 52.674' N, 120° 45.736' W, 4414 ft., *Abies concolor* var. *concolor* / *lowiana*: Adams 12522-12526, on CA Hwy 38 north side of Onyx Summit, CA, 34° 12.037' N, 116° 43.520' W, 8490 ft. All specimens are deposited in the BAYLU herbarium.

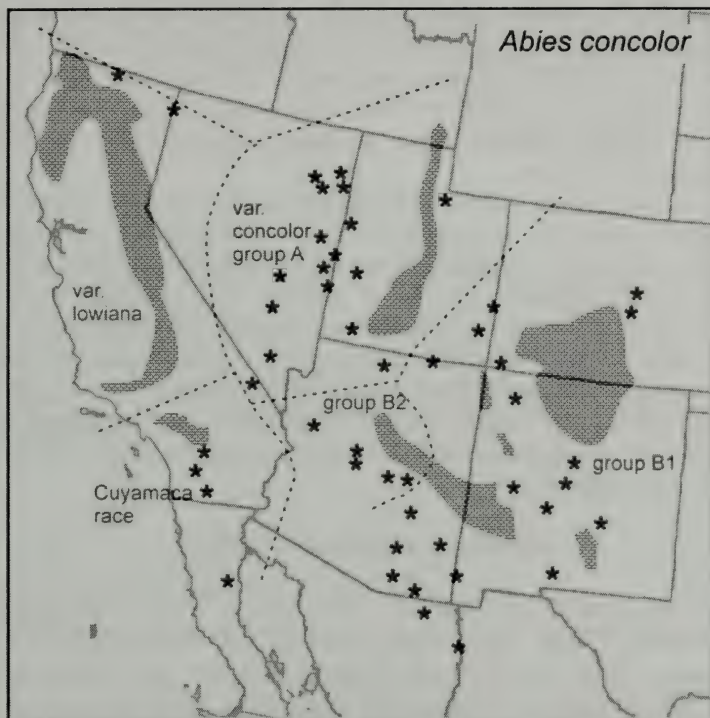


Figure 1. Distribution of *Abies concolor* (modified from Zavarin et al. 1975) with subgroups based on wood monoterpene data.

*Isolation of Oils* - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

*Chemical Analyses* - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on

a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as percent total oil) were coded and compared among the species by the Gower (1971) metric. Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

## RESULTS AND DISCUSSION

In general, the leaf oils of *A. concolor* are dominated by monoterpenes with only small amounts of sesquiterpenes and diterpenes (Table 1). Each of the populations are high in  $\beta$ -pinene, except the Cimarron, NM site where  $\beta$ -pinene is 3.1% (Table 1). The populations from central and northern California (Lee S, Plac, Klam, Table 1) share several components at similar levels: linalool,  $\alpha$ -terpineol, geranyl acetate, RI 1617 sesquiterpene alcohol, and eudesm-7(11)-en-4-ol and all are devoid of (E)- $\beta$ -ocimene and 6-methyl-5-octen-one (Table 1). The New Mexico oil is the most unusual oil being high in camphene (25.9%), bornyl acetate (28.7) and  $\alpha$ -pinene (11.2) with several unique components: germacrene D,  $\alpha$ -muurolene,  $\alpha$ -acorenol, germacrene-4(15),5,10(14)-trien-1-al, benzyl benzoate and cembrene (Table 1).

The overall similarities of the oils are shown in figure 2. Notice *A. c.* var. *lowiana* from central and northwestern California have very similar oils (0.833, 0.912, Fig. 2). The major difference in the



Klamath Mtns. oil is the presence of intermedeol (6.45, Table 1) that was only found in this oil. The oils from Utah and Onyx Summit are the next most similar (0.764, Fig. 2), with the oil from New Mexico being the least similar (0.670, Fig. 2).

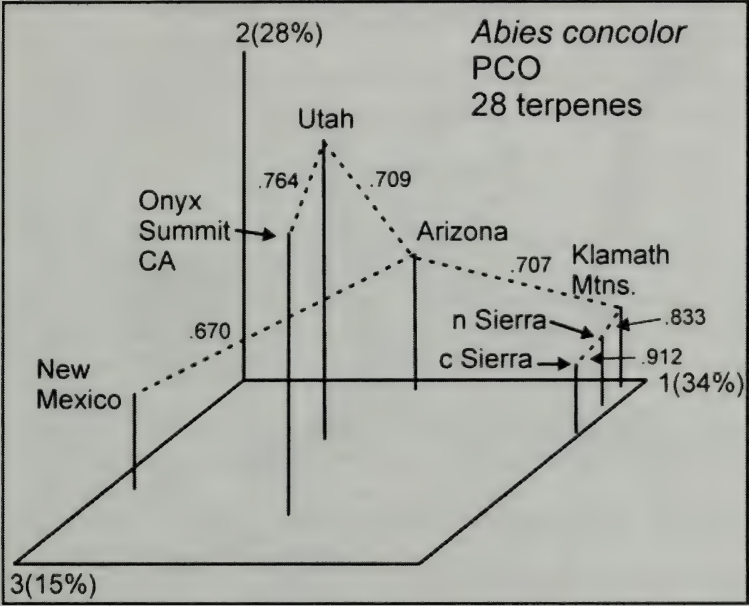


Figure 2. PCO based on 28 terpenes. The dotted lines are the minimum spanning network and the numbers next to the lines are the similarities.

Mapping the minimum spanning network onto the distribution of *Abies concolor* (Fig. 3) clearly shows the geographic affinities. The unity of the central and northwestern California *A. c.* var. *lowiana* populations is clear. Zavarin et al. (1975) designated the southern California populations as the Cuyamaca race and this analysis confirms their observation. Zavarin et al. (1975) did not indicate a strong affinity (in the wood monoterpenes) of the Cuyamaca Race to var. *concolor*, group A, but there is a high similarity in their oils (Figs. 2, 3).

Zavarin et al. (1975) divided var. *concolor* into 3 sub-groups: A, B1 and B2. The present analysis (based on leaf essential oils) confirms the same pattern of differentiation (Figs. 2, 3).

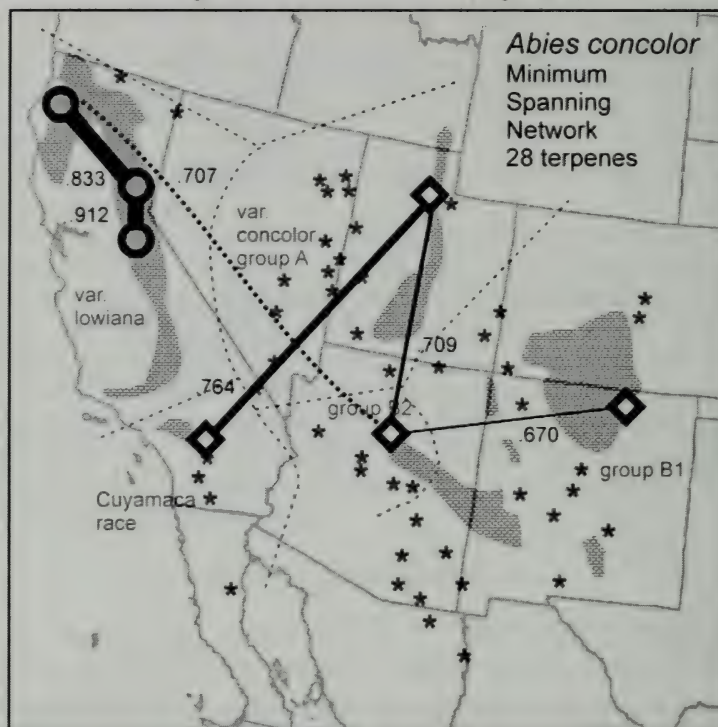


Figure 3. Minimum spanning network based on 20 terpenes. The open circles are *A. c. var. lowiana*, the open squares are generally treated as *A. c. var. concolor*. The numbers next to the lines are similarities.

## CONCLUSIONS

In general there was very good agreement in the pattern of differentiation with Zavarin et al. (1975). The differentiation of the New Mexico oils was much greater than Zavarin et al. (1975) found in the wood monoterpenes. Additional sampling is needed (in progress, RPA) of *A. concolor* from Colorado, Utah and eastern Arizona to more clearly demarcate this differentiation.

It is premature to make taxonomic decisions at this time. Molecular research is needed (RPA, in progress) to clarify the taxonomic relationships of the varieties, the Cuyamaca Race and the unusual New Mexico plants.

### ACKNOWLEDGEMENTS

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Table 1. Comparison of leaf oil compositions of *Abies concolor*. Klam = Klamath Mtns., NW CA, Lee S = Lee Summit, CA, Plac = Placerville, CA, AZ = Pine, AZ, NM = Cimarron, New Mexico, UT = Wasatch Mtns., UT, Onyx = Onyx Summit, CA. Compounds in bold face appear to separate the taxa. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. RI is the Kovat's Index using a linear approximation on DB-5 column. \* = cpds used for PCO (28 cpds.)

RI	compound	Lee S	Plac	Klam	AZ	UT	Onyx	NM
884	santene*	0.2	0.2	0.3	-	t	t	1.6
921	tricyclene*	0.2	0.1	0.2	0.3	0.2	t	2.5
924	$\alpha$ -thujene	t	t	t	t	t	t	-
<b>932</b>	<b><math>\alpha</math>-pinene*</b>	<b>5.1</b>	<b>4.4</b>	<b>4.7</b>	<b>7.9</b>	<b>8.9</b>	<b>7.8</b>	<b>11.2</b>
<b>946</b>	<b>camphene*</b>	<b>2.5</b>	<b>1.4</b>	<b>2.7</b>	<b>4.3</b>	<b>2.9</b>	<b>0.5</b>	<b>25.9</b>
969	sabinene	t	t	t	t	t	t	0.1
<b>974</b>	<b><math>\beta</math>-pinene*</b>	<b>42.0</b>	<b>47.1</b>	<b>45.2</b>	<b>52.2</b>	<b>43.9</b>	<b>41.5</b>	<b>3.1</b>
988	myrcene*	2.0	2.2	1.4	2.2	2.1	1.7	2.0
1002	$\alpha$ -phellandrene	0.4	0.4	0.3	0.3	0.2	0.4	0.1
1008	$\delta$ -3-carene*	0.1	0.1	0.2	0.5	1.6	1.1	0.8
1014	$\alpha$ -terpinene	0.2	0.2	0.2	0.1	0.1	0.2	t
1020	p-cymene	t	t	t	t	t	t	t
<b>1024</b>	<b>limonene*</b>	<b>23.0</b>	<b>23.0</b>	<b>17.6</b>	<b>9.0</b>	<b>9.3</b>	<b>21.2</b>	<b>9.1</b>
1025	$\beta$ -phellandrene	2.5	2.5	2.0	1.1	1.1	2.5	1.2
1032	(Z)- $\beta$ -ocimene	t	-	t	0.3	0.2	0.1	t
1038	2-heptyl acetate	-	-	-	-	t	-	-



RI	compound	Lee S	Plac	Klam	AZ	UT	Onyx	NM
1044	(E)- $\beta$ -ocimene*	-	-	-	0.3	0.3	0.1	0.5
1054	$\gamma$ -terpinene	0.2	0.2	0.1	0.2	0.2	0.2	0.2
1077	(6-methyl-5-octen-2-one)	-	-	-	0.1	0.1	0.1	-
1086	terpinolene*	1.4	1.7	1.0	0.7	1.7	1.4	1.2
1087	2-nonanone*	0.3	0.4	0.1	0.5	0.6	0.4	0.1
1095	linalool*	0.4	0.2	0.1	1.4	1.8	1.4	3.1
1118	endo-fenchol*	0.4	0.4	0.3	0.2	1.8	1.6	t
1118	cis-p-menth-2-en-1-ol	0.4	0.4	0.3	0.1	0.2	0.4	t
1122	$\alpha$ -campholenal	0.1	0.1	t	0.1	0.2	0.2	t
1136	trans-p-menth-2-en-1-ol	0.3	0.3	0.1	t	0.1	0.3	t
1141	camphor	0.1	t	t		0.1	t	0.2
1145	camphene hydrate*	0.3	0.2	0.1	1.8	4.1	0.5	1.2
1148	citronellal*	0.1	0.1	0.3	0.6	0.3	0.2	0.2
1155	iso-borneol	t	t	t	t	0.1	t	t
1165	borneol*	0.5	0.3	0.2	0.2	0.6	0.8	1.5
1172	cis-pinocamphone	t	t	t	t	-	t	t
1174	terpinen-4-ol*	0.4	0.4	0.3	0.3	0.6	0.7	0.8
1183	cryptone	t	t	t	t	-	t	-
1186	$\alpha$ -terpineol*	6.5	6.9	4.8	2.2	3.5	4.5	0.8
1195	cis-piperitol	0.2	0.2	0.1	t	t	0.1	t
1201	n-decanal	0.1	0.3	-	0.2	0.1	0.2	t

RI	compound	Lee S	Plac	Klam	AZ	UT	Onyx	NM
1207	trans-piperitol	0.2	0.1	t	t	t	0.1	-
1218	endo-fenchyl acetate	-	t	t	0.1	t	t	t
1223	citronellol*	0.5	0.2	0.3	0.4	0.6	1.0	0.3
1235	neral	0.3	0.1	-	-	t	t	-
1249	<b>piperitone*</b>	<b>0.4</b>	<b>0.1</b>	<b>t</b>	<b>t</b>	<b>1.1</b>	<b>1.7</b>	<b>0.2</b>
1264	geranial	0.6	0.4	-	t	0.2	0.2	t
1287	<b>bornyl acetate*</b>	<b>2.8</b>	<b>1.2</b>	<b>6.6</b>	<b>8.8</b>	<b>6.4</b>	<b>0.6</b>	<b>28.7</b>
1289	<b>thymol*</b>	<b>t</b>	<b>t</b>	<b>-</b>	<b>t</b>	<b>1.3</b>	<b>0.6</b>	<b>-</b>
1293	2-undecanone	0.1	0.3	0.2	0.4	0.1	0.4	-
1300	tridecane*	0.2	0.1	t	0.3	0.2	0.8	-
1350	citronellyl acetate*	0.2	0.2	0.2	0.5	t	0.1	0.4
1374	$\alpha$ -copaene	-	-	-	-	-	-	0.1
1379	geranyl acetate*	0.5	0.8	0.4	0.2	t	t	0.3
1395	sesquiterpene, 43, 55, 86, 206	0.1	0.1	-	0.3	t	-	t
1408	dodecanal	t	t	-	t	t	0.1	-
1417	(E)-caryophyllene	t	t	t	t	0.2	0.3	t
1478	$\gamma$ -muurolene	0.1	t	0.2	-	-	t	-
1480	<b>germacrene D*</b>	-	-	-	-	-	-	<b>0.2</b>
1496	valencene	0.2	0.2	0.3	t	-	0.2	-
1500	<b><math>\alpha</math>-muurolene</b>	-	-	-	-	-	-	<b>0.1</b>
1514	cubebol	-	-	0.2	0.1	-	0.3	-

RI	compound	Lee S	Plac	Klam	AZ	UT	Onyx	NM
1522	$\delta$ -cadinene	-	-	t	0.1	-	0.4	0.1
1559	germacrene B	0.1	t	-	t	-	t	-
1561	(E)-nerolidol	0.1	t	-	t	0.1	t	-
<b>1617</b>	<b>sesquiterpene, 81, 161,</b>							
<b>189, 222*</b>		<b>0.5</b>	<b>0.3</b>	<b>0.4</b>	<b>t</b>	<b>t</b>	-	-
1627	1-epi-cubenol	0.1	t	-	t	-	0.3	-
1630	$\alpha$ -acorenenol	-	-	-	-	-	-	0.1
1649	$\beta$ -eudesmol	0.2	0.1	-	-	t	-	-
1652	$\alpha$ -eudesmol	0.2	0.1	-	-	t	-	-
1652	$\alpha$ -cadinol	0.2	0.1	-	-	t	-	0.4
<b>1665</b>	<b>intermedeol*</b>	-	-	<b>6.4</b>	-	-	-	-
<b>1685</b>	<b>germacra-4(15), 5, 10(14)-</b>							
	<b>trien-1-al</b>	-	-	-	-	-	-	<b>0.2</b>
1700	eudesm-7(11)-en-4-ol	0.1	0.1	0.1	-	t	-	-
<b>1756</b>	<b>benzyl benzoate</b>	-	-	-	-	-	-	<b>0.1</b>
<b>1937</b>	<b>cembrene</b>	-	-	-	-	-	-	<b>0.1</b>
1987	manoyl oxide	t	-	t	0.1	t	t	t
2014	palustradiene(=abieta-8, 13-diene) *	0.1	t	t	t	t	0.3	t
2056	manool	0.1	0.1	t	t	t	t	t
2149	abienol	0.1	0.1	t	t	t	0.2	t

**SYSTEMATICS OF *JUNIPERUS CHINENSIS* AND *J. TSUKUSIENSIS* FROM JAPAN AND TAIWAN: DNA SEQUENCING AND TERPENOID.**

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**ABSTRACT**

Analyses of nrDNA, petN-psbM, trnD-trnT and trnS-trnG revealed that *Juniperus chinensis* var. *tsukusiensis* and *J. c.* var. *taiwanensis* are not conspecific with *J. chinensis*. In addition, analyses of the leaf oils (terpenoids) also revealed numerous differences. Based on these new data, *J. c.* var. *tsukusiensis* is recognized as *J. tsukusiensis* Masam. and *J. c.* var. *taiwanensis* as ***J. tsukusiensis* var. *taiwanensis* (R. P. Adams and C-F. Hsieh) R. P. Adams, comb. nov.** *Phytologia* 93(1): 118-131 (April 1, 2011).

**KEY WORDS:** *Juniperus chinensis*, *J. tsukusiensis*, *J. tsukusiensis* var. *taiwanensis*, *J. jarkendensis*, DNA, terpenoids, systematics.

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Adams et al. (2002) examined the RAPDs from putative *J. chinensis* from Japan and Taiwan and found (Fig. 1) that *J. chinensis* L. (Japan) was quite distinct from *J. c.* var. *sargentii* Henry (both high and low bornyl acetate types), and *J. c.* var. *tsukusiensis* Masam. (Yakushima, Japan) and *J. c.* var. *taiwanensis* R. P. Adams and C-F. Hsieh (Taiwan). Notice that *J. c.* var. *taiwanensis* was well resolved from *J. chinensis* and *J. c.* var. *tsukusiensis* (Fig. 1).

In his newest monograph of *Juniperus* (Adams, 2011) recognized *J. chinensis* with three varieties: var. *sargentii*, Japan, var. *taiwanensis*, endemic to Mt. Chingshui, Taiwan, and var. *tsukusiensis*., endemic to the off shore island of Yaku Shima, Japan.

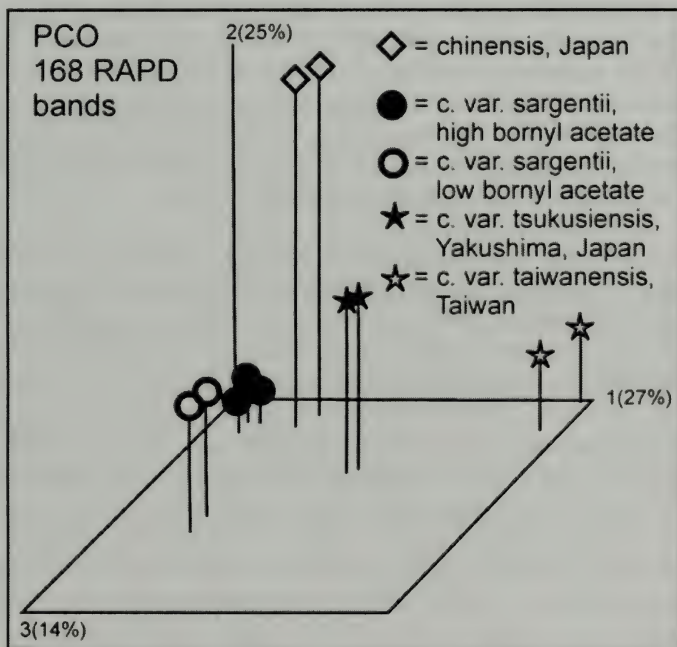


Figure 1. PCO based on 168 RAPD bands for *J. chinensis* taxa from Japan and Taiwan (adapted from Adams et al., 2002).

Recent DNA sequencing in our labs indicate that *J. c.* var. *taiwanensis* and var. *tsukusiensis* are more closely related to *J.*

*jarkendensis* than to *J. chinensis*. In order to understand the relations, we have sequenced additional regions and also analyzed the leaf terpenoids. The purpose of this paper is to present the sequencing and leaf oil analyses to resolve the relationships of *J. c. var. taiwanensis* and *var. tsukusiensis* to other *J. chinensis* taxa.

## MATERIALS AND METHODS

Specimens collected: *J. chinensis*, Adams8535-8537, Shizuoka Prefecture, Osezaki Point, 3m, Japan, 16 June 1998, *J. c. var. sargentii*, Adams 8688, collected by Naotoshi Yoshida at the Medicinal Bot. Gard., Hokkaido Univ., Japan, *J. c. var. taiwanensis*, Adams 9061-9063, Mt. Chingshui, *ex situ* Taiwan Forestry Institute, 24 June 2000, *J. c. var. tsukusiensis*, Adams 8805-8808, collected by K. Miyazaki via Jin Murata, Mt Kuromidake, 1500m, Yaku Shima, Japan, 4 Aug. 1999; *J. jarkendensis*, Adams 7820-7825, Kunlun Mtns., 2600 m, Oetak, above Akto forestry station, Xinjiang, China, 28 July 1996; *J. occidentalis*, Adams 8592-8594, 0.2 km nw of Sisters, OR, USA, 17 Oct. 1998. Voucher specimens are deposited at BAYLU.

*Isolation of Oils* - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

*Chemical Analyses* - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by the Gower (1971) metric. Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

DNA Analysis - One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at  $-20^{\circ}\text{C}$  until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA). PCR amplifications were performed in 30  $\mu\text{l}$  reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15  $\mu\text{l}$  2x buffer E (petN-psbM, trnDT, trnSG) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200  $\mu\text{M}$  each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM  $\text{MgCl}_2$  according to the buffer used) 1.8  $\mu\text{M}$  each primer. See Adams et al. (2011) for the ITS, petN-psbM, trn D-trnT and trnS-trnG primers utilized. The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/>). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

## RESULTS AND DISCUSSION

The leaf oils exhibited considerable differences among the taxa (Table 1). *Juniperus chinensis* (Japan) was dominated by sabinene (27.5%), and bornyl acetate (19.7%) with moderate amounts of myrcene (5.5%), limonene (6.1%),  $\beta$ -phellandrene (4.1%) and elemol (6.1%).

This oil differs from the others by having pregeijerene B, (E)-caryophyllene, cis-cadina-1,4-diene, epi-zonarene, 10-epi-cubebol and 8- $\alpha$ -acetoxylemol (Table 1). The amount of bornyl acetate is polymorphic with a range of 2.5 to 30.2%.

The oils of *J. c.* var. *tsukusiensis* and var. *taiwanensis* are very similar (Table 1). Both have large amounts of  $\alpha$ -pinene (33.2, 13.4%), sabinene (11.5, 1.4%), myrcene (5.6, 11.6%), bornyl acetate (8.4, 22.5%),  $\delta$ -cadinene (5.2, 4.0%) and  $\alpha$ -cadinol (4.7, 7.4%). These two taxa share ten compounds not found in the other taxa:  $\alpha$ -copaene,  $\beta$ -cubebene, trans-muurolo-3,5-diene, trans-muurolo-4(14),5-diene, trans-cadina-1,4-diene,  $\alpha$ -cadinene,  $\beta$ -oplophenone, 1-epi-cubenol,  $\alpha$ -muurolol and  $\alpha$ -cadinol. The oil of *J. c.* var. *taiwanensis* had no unique components (greater than a trace) and var. *tsukusiensis* had one component (naphthalene). The amount of bornyl acetate was nearly constant in var. *taiwanensis* ranging from 6.2 to 9.6 %, but wide ranging in var. *tsukusiensis* from 11.7 to 32.3%.

The oil of *J. jarkendensis* is very different from the other oils (Table 1) and is dominated by sabinene (57.7%) and cedrol (9.1%). The presence of cedrol (a major component of *Juniperus* wood oils, Adams, 1991, 2009; Adams and Lu, 2008) is found in the leaf oils of only a few species in the world (Adams, 2011). Several other typical wood oil components were present:  $\alpha$ -cedrene,  $\beta$ -cedrene, cis-thujopsene, allo-cedrol and cedryl acetate. It seems likely that the pathway to these compounds is activated in the leaves of *J. jarkendensis*, along with the typical leaf oil components. This makes the oil appear very different from the other taxa (Table 1). Aside from the 'wood oil' components, the leaf oil is still quite different in having cis- and trans-thujone, methyl citronellate, trans-sabinyol acetate, methyl geranate, as well as lacking in sesquiterpenes.

Overall, the oils of *J. c.* var. *taiwanensis* and var. *tsukusiensis* are very similar but differ from *J. chinensis* and *J. jarkendensis* oils.

NJ analyses, based on combined nrDNA, petN-psbM, trnD-trnT and trnS-trnG sequences, is shown in Figure 2. There is support for the separate clades of (*J. jarkendensis*, *J. c.* var. *taiwanensis*, *J. c.* var. *tsukusiensis*) and (*J. chinensis*, *J. c.* var. *sargentii*). There is also support



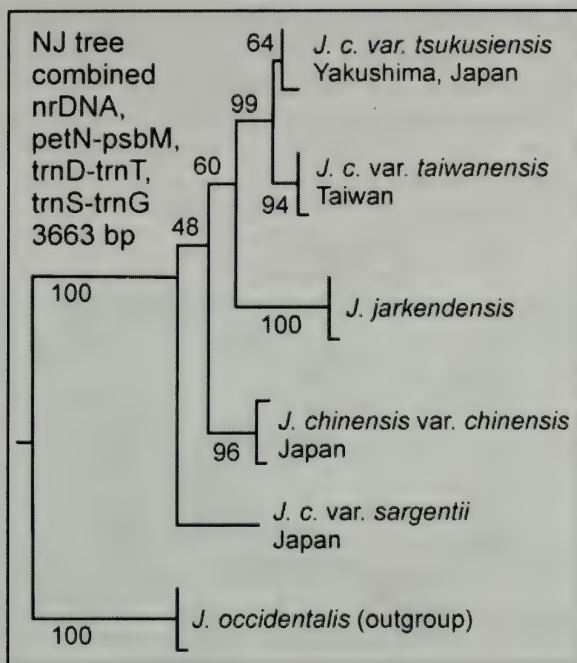


Figure 2. NJ tree based on combined sequence data. The numbers are bootstrap percentages (1000 reps).

for *J. c. var. taiwanensis* and *J. c. var. tsukusiensis* belonging to separate clades. Of course, merely being in separate clades does not indicate if these taxa are distinct species or varieties. One should note that parsimony analysis gave conflicting trees which appears to be due to the inconsistent evolution among the data sets (see SNPs analyses below).

A different method to view the sequence data is by utilizing SNPs (including indel information). Figure 3 shows minimum spanning networks based on nrDNA and petN-psbM. The SNPs from nrDNA show *J. chinensis var. chinensis*, *J. c. var. taiwanensis* and *J. c. var. tsukusiensis* differ by only one SNP (Fig. 3, left). Interestingly, *J. c. var. sargentii* differs from *J. c. var. chinensis* by 7 SNPs which is greater than the 5 SNPs that separate *J. jarkendensis* from *J. c. var. taiwanensis* and var. *tsukusiensis* (Fig. 3, left).

The pattern for petN-psbM SNPs (including indel data) (Fig. 3, right) is quite different as both *J. c. var. taiwanensis* and var. *tsukusiensis* are shown more related to *J. jarkendensis* than to each other or to *J. chinensis*. *Juniperus chinensis* var. *sargentii* differs by 10 SNPs from *J. jarkendensis* but by only 1 SNP from *J. c. var. chinensis* (Fig. 3, right).

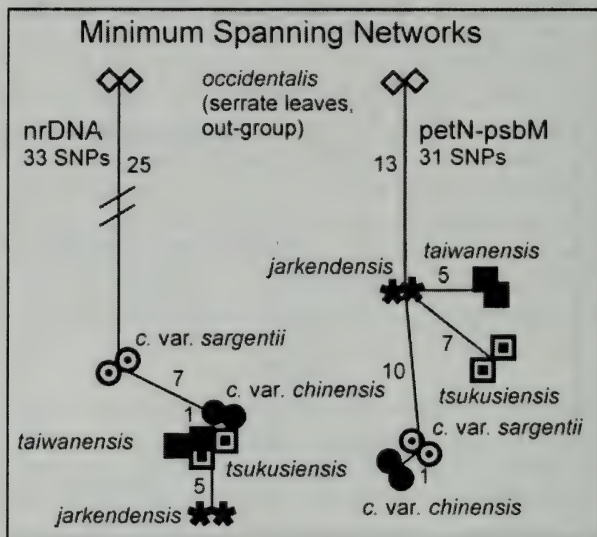


Figure 3. Minimum spanning networks based on nrDNA and on petN-psbM. The numbers next to lines are the number of SNPs.

The SNPs from trnD-trnT (Fig. 4, left) show a similar pattern as seen for petN-psbM (Fig. 3, left) in that *J. c. var. chinensis* and var. *sargentii* differ by only one SNP. However, *J. c. var. taiwanensis* and var. *tsukusiensis* are nearly identical (1 SNP, Fig. 4, left) and only 2 SNPs removed from *J. jarkendensis*.

The evolution within trnS-trnG (Fig. 4, right) is similar to the pattern of trnD-trnT in that *J. c. var. chinensis* and var. *sargentii* have no differences and *J. c. var. taiwanensis* and var. *tsukusiensis* are nearly identical (1 SNP, Fig. 4, right). *Juniperus jarkendensis* is much more distinct (5 SNPs, Fig. 4) than seen in analysis of trnD-trnT (Fig. 4, left).

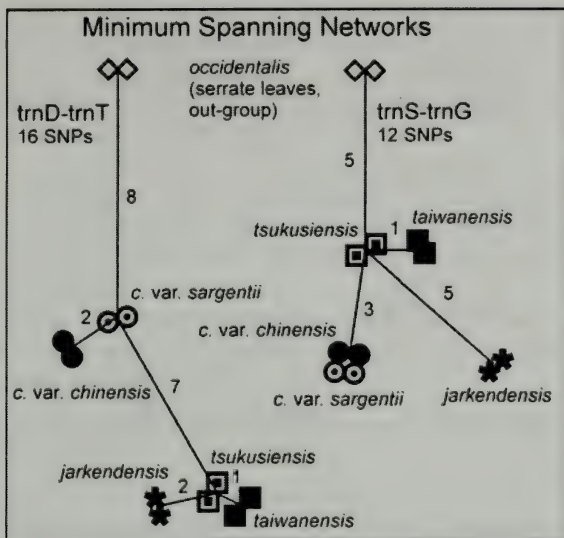


Figure 4. Minimum spanning networks based on trnD-trnT and trnS-trnG.

The overall minimum spanning network based on 92 SNPs shows considerable differentiation between *J. c. var. chinensis* and var. *sargentii* (10 SNPs, Fig. 5) and *J. c. var. taiwanensis* and var. *tsukusiensis* (10 SNPs, Fig. 5). The *chinensis-sargentii* group is separated by 24 SNPs from the *taiwanensis-tsukusiensis* group (Fig. 5). The *taiwanensis-tsukusiensis* group is a little closer to *J. jarkendensis* (19 SNPs, Fig. 5) than the *chinensis-sargentii* group (24 SNPs, Fig. 5).

The finding by DNA sequencing that the Yaku Shima and Taiwan junipers are not as closely related to *J. chinensis* (Japan) as to *J. jarkendensis* (w. China) was unexpected. The leaf oils are more like *J. chinensis* than *J. jarkendensis* (Table 1). Adams (2011) noted that *J. c. var. taiwanensis* and var. *tsukusiensis* differ from *J. chinensis* in being procumbent shrubs, with scale leaves that are very short and wide (appearing as a sting of beads), and with glands that are raised (vs. sunken in *J. chinensis*).

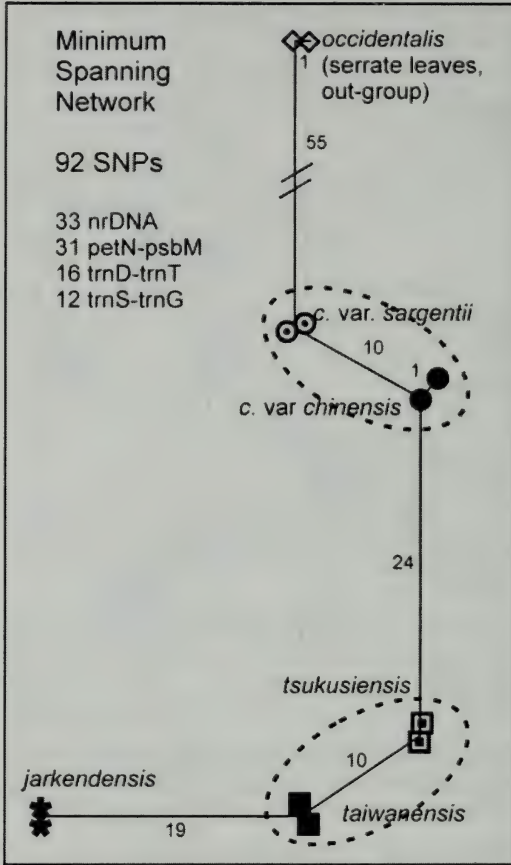


Figure 5. Minimum spanning network based on combined data from four sequences. Numbers on lines are the number of SNPs (including indels).

Considering all the data available at present, it seems prudent to follow Masamune's original species concept [Bot. Mag. Tokyo 44: 50 (1930)] and recognize *J. c. var. tsukusiensis* (Masam.) Masam. as a distinct species: ***J. tsukusiensis***, Type: Japan, Yaku Shima, G. Masamune s. n. (syntype IT), known only from steep rocks on Yaku Shima. In addition, the relationship between var. *tsukusiensis* and var. *taiwanensis* seems, at present, appropriately characterized as being conspecific at the



variety level. This warrants the recognition and moving of *J. c.* var. *taiwanensis* to a variety of *J. tsukusiensis* as:

*Juniperus tsukusiensis* Masam. var. *taiwanensis* (R. P. Adams and C-F. Hsieh) R. P. Adams, **comb. nov.**

**Basionym:** *Juniperus chinensis* L. var. *taiwanensis* R. P. Adams and C-F. Hsieh (Taiwan). Biochem. Syst. Ecol. 30: 235 (2002), Taiwan juniper, Type: Taiwan, Mt. Chingshui, 200 m, Sheng-you Lu 14498 (HOLOTYPE: TAIF).

Distribution: Known only from the type locality, about 100 m below the summit of Mt. Chingshui, Taiwan. The currently recognized distribution of *J. tsukusiensis* is shown in Figure 6.



Figure 6. Distribution of *J. tsukusiensis* var. *tsukusiensis* (endemic to Yakushima) and *J. t.* var. *taiwanensis* (endemic to Taiwan).

## ACKNOWLEDGEMENTS

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Table 1. Comparison of leaf essential oils of *J. chinensis* (Chin), *J. c. var. taiwanensis* (Taiw), *J. c. var. tsukusiensis* (Tsuk) and *J. jarkendensis* (Jark). Compounds in bold appear to separate the taxa. t = trace, < 0.1%, RI = retention index on DB-5.

RI	Component	Chin	Taiw	Tsuk	Jark
921	tricyclene	0.9	0.4	0.8	t
924	$\alpha$ -thujene	0.9	0.4	t	1.3
<b>932</b>	<b><math>\alpha</math>-pinene</b>	<b>1.8</b>	<b>33.2</b>	<b>13.4</b>	<b>2.5</b>
946	camphene	0.9	0.7	0.8	0.1
<b>969</b>	<b>sabinene</b>	<b>27.5</b>	<b>11.5</b>	<b>1.4</b>	<b>57.7</b>
<b>974</b>	<b><math>\beta</math>-pinene</b>	<b>0.3</b>	<b>4.0</b>	<b>1.8</b>	<b>t</b>
<b>988</b>	<b>myrcene</b>	<b>5.5</b>	<b>5.6</b>	<b>11.6</b>	<b>3.1</b>
1001	$\delta$ -2-carene	0.1	0.8	0.2	-
1002	$\alpha$ -phellandrene	-	-	t	0.1
1008	$\delta$ -3-carene	-	t	-	t
1014	$\alpha$ -terpinene	0.7	0.4	0.1	1.2
1020	p-cymene	0.1	t	t	0.6
1024	limonene	6.1	2.6	3.0	1.7
1025	$\beta$ -phellandrene	4.1	2.6	3.0	0.4
1044	(E)- $\beta$ -ocimene	0.4	-	t	0.2
1054	$\gamma$ -terpinene	1.0	0.6	0.2	2.0
1065	cis-sabinene hydrate	0.6	0.2	0.1	1.0
1086	terpinolene	1.0	0.6	0.5	0.9
<b>1096</b>	<b>trans-sabinene hydrate</b>	<b>0.2</b>	<b>0.1</b>	-	<b>0.6</b>
<b>1097</b>	<b>linalool</b>	<b>1.6</b>	<b>0.2</b>	-	<b>1.1</b>
1100	n-nonanal	-	t	t	-
<b>1101</b>	<b>cis-thujone</b>	-	-	-	<b>0.2</b>
1102	isopentyl-isovalerate	-	-	t	-
<b>1112</b>	<b>trans-thujone</b>	-	-	-	<b>1.6</b>
1112	3-methyl-3-buten-methyl- butanoate	-	-	t	-
1118	cis-p-menth-2-en-1-ol	0.1	0.1	t	0.3
1134	iso-3-thujanol	-	-	-	0.1
1136	trans-p-menth-2-en-1-ol	t	t	t	0.3
<b>1141</b>	<b>camphor</b>	<b>0.2</b>	<b>0.2</b>	<b>0.9</b>	-
1145	camphene hydrate	0.1	t	t	-
1148	citronellal	-	-	-	0.1
1154	sabina ketone	-	-	-	0.1
1155	isoborneol	-	-	t	-
1165	borneol	0.2	0.1	1.1	-
1174	terpinen-4-ol	0.2	1.0	0.7	4.9

RI	Component	Chin	Taiw	Tsuk	Jark
<b>1178</b>	<b>naphthalene</b>	-	-	<b>0.5</b>	-
1186	$\alpha$ -terpineol	0.1	0.1	0.5	0.2
1195	cis-piperitol	-	t	-	0.1
1207	trans-piperitol	-	t	-	0.1
1218	endo-fenchyl acetate	-	t	-	-
1219	coahuilensol	-	-	-	0.1
<b>1223</b>	<b>citronellol</b>	-	<b>0.1</b>	<b>t</b>	<b>0.8</b>
1235	neral	-	-	-	t
1249	piperitone	-	t	t	-
1253	trans-sabinene hydrate acetate	-	-	-	0.1
<b>1257</b>	<b>methyl citronellate</b>	-	-	-	<b>1.2</b>
1260	3-methyl-3-butenol, hexanoate	- t	- -	- t	- -
<b>1274</b>	<b>pregeijerene B</b>	<b>1.5</b>	-	-	-
<b>1287</b>	<b>bornyl acetate</b>	<b>19.7</b>	<b>8.4</b>	<b>22.5</b>	<b>0.1</b>
<b>1289</b>	<b>trans-sabinyl acetate</b>	-	-	-	<b>2.7</b>
<b>1322</b>	<b>methyl geranate</b>	-	-	-	<b>0.8</b>
1345	$\alpha$ -cubebene	-	t	t	-
<b>1374</b>	<b><math>\alpha</math>-copaene</b>	-	<b>0.1</b>	<b>0.1</b>	-
1380	daucene	-	t	-	-
<b>1387</b>	<b><math>\beta</math>-cubebene</b>	-	<b>0.1</b>	<b>0.1</b>	-
<b>1410</b>	<b><math>\alpha</math>-cedrene</b>	-	-	-	<b>0.5</b>
<b>1417</b>	<b>(E)-caryophyllene</b>	<b>0.1</b>	-	-	-
<b>1419</b>	<b><math>\beta</math>-cedrene</b>	-	-	-	<b>0.2</b>
1429	cis-thujopsene	-	-	-	0.1
1448	cis-muurolo-3,5-diene	0.6	0.1	0.1	-
<b>1451</b>	<b>trans-muurolo-3,5-diene</b>	-	<b>0.1</b>	<b>0.1</b>	-
1452	$\alpha$ -humulene	0.2	t	-	-
1461	cis-cadina-1(6),4-diene	-	-	-	-
1465	cis-muurolo-4(14),5-diene	1.3	0.3	0.4	-
1475	trans-cadina-1(6),4-diene	-	-	0.2	-
1478	$\gamma$ -muurolene	-	-	0.4	-
1480	germacrene D	0.3	0.2	0.1	-
<b>1493</b>	<b>trans-muurolo-4(14),5- diene</b>	-	<b>0.1</b>	<b>0.3</b>	-
1493	epi-cubebol	0.1	0.4	0.5	-
1495	epi-cubebene	-	-	-	-
<b>1495</b>	<b>cis-cadina-1,4-diene</b>	<b>0.1</b>	-	-	-
<b>1500</b>	<b>epi-zonarene</b>	<b>0.1</b>	-	-	-



RI	Component	Chin	Taiw	Tsuk	Jark
<b>1501</b>	<b><math>\alpha</math>-muurolene</b>	<b>0.1</b>	<b>1.4</b>	<b>1.2</b>	-
<b>1513</b>	<b><math>\gamma</math>-cadinene</b>	<b>0.2</b>	<b>1.2</b>	<b>1.4</b>	-
<b>1513</b>	<b>cubebol</b>	<b>0.1</b>	<b>0.5</b>	<b>0.7</b>	-
1522	$\delta$ -cadinene	1.1	5.2	4.0	0.1
<b>1533</b>	<b>10-epi-cubebol</b>	<b>1.7</b>	-	-	-
<b>1533</b>	<b>trans-cadina-1,4-diene</b>	-	<b>0.2</b>	<b>0.1</b>	-
<b>1537</b>	<b><math>\alpha</math>-cadinene</b>	-	<b>0.4</b>	<b>0.4</b>	-
<b>1548</b>	<b>elemol</b>	<b>6.1</b>	<b>t</b>	-	<b>0.2</b>
1550	cis-muurola-5-en-4- $\beta$ -ol	-	-	t	-
1559	cis-muurola-5-en-4- $\alpha$ -ol	0.5	t	t	-
1559	germacrene B	-	-	-	t
1574	germacrene-D-4-ol	0.8	3.7	6.8	0.1
<b>1589</b>	<b>allo-cedrol</b>	-	-	-	<b>0.4</b>
<b>1600</b>	<b>cedrol</b>	-	-	-	<b>9.1</b>
<b>1607</b>	<b><math>\beta</math>-oplophenone</b>	-	<b>0.5</b>	<b>0.9</b>	-
1618	1,10-di-epi-cubebol	1.7	0.1	0.1	-
<b>1627</b>	<b>1-epi-cubenol</b>	-	<b>0.2</b>	<b>0.2</b>	-
1630	$\gamma$ -eudesmol	0.4	-	-	-
1638	epi- $\alpha$ -cadinol	0.2	1.8	1.1	t
1638	epi- $\alpha$ -muurolol	0.3	1.7	4.2	t
<b>1644</b>	<b><math>\alpha</math>-muurolol</b>	-	<b>0.7</b>	<b>1.0</b>	-
1649	$\beta$ -eudesmol	0.6	-	-	t
1652	$\alpha$ -eudesmol	0.7	-	-	-
<b>1652</b>	<b><math>\alpha</math>-cadinol</b>	<b>1.5</b>	<b>4.7</b>	<b>7.4</b>	<b>0.1</b>
1670	bulnesol	0.5	-	-	-
1688	shyobunol	-	-	t	-
<b>1767</b>	<b>cedryl acetate</b>	-	-	-	<b>0.1</b>
<b>1792</b>	<b>8-<math>\alpha</math>-acetoxylemol</b>	<b>0.8</b>	-	-	-
1958	iso-pimara-8(14),15-diene	0.2	t	-	-
1988	manoyl oxide	0.2	0.3	-	t
2055	abietatriene	0.3	0.1	0.1	t
2087	abietadiene	t	t	-	t
<b>2282</b>	<b>sempervirol</b>	<b>0.8</b>	<b>0.2</b>	<b>2.1</b>	-
2298	4-epi-abietal	0.3	0.3	0.3	0.1
<b>2314</b>	<b>trans-totarol</b>	<b>0.4</b>	<b>0.1</b>	<b>0.9</b>	-
<b>2331</b>	<b>trans-ferruginol</b>	<b>0.1</b>	<b>t</b>	<b>0.2</b>	-

## THE TAXNOMIC AFFINITY OF A JUNIPER POPULATION FROM COLONIA PACHECO, MEXICO

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### ABSTRACT

The taxonomic affinity of junipers from Colonia Pacheco was examined using volatile leaf oils, nrDNA SNPs, morphology, and ecology. The leaf oil was compared with oils from *J. scopulorum* (Durango, CO), *J. blancoi*, *J. b.* var. *huehuentensis* and *J. b.* var. *mucronata*. The juniper population at Colonia Pacheco appears to be the northern-most known population of *J. blancoi* with its leaf oil containing a few components characteristic of *J. scopulorum* suggesting gene exchange in the Pleistocene. *Phytologia* 93(1): 132-145 (April 1, 2011).

**KEY WORDS:** *Juniperus scopulorum*, *J. blancoi*, *J. b.* var. *huehuentensis*, *J. b.* var. *mucronata*, leaf terpenoids, geographic variation, nrDNA, SNPs, Pleistocene refugia.

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In a previous study, Adams (2011) reported that the leaf volatile oil of putative *Juniperus scopulorum* Sarg. from Colonia Pacheco, Mexico was very divergent from typical *J. scopulorum* in the Rocky Mountains. In an effort to further analyze the affinities of this divergent population, the composition of the volatile leaf oils of *J. scopulorum*, Durango, CO, *J. blancoi* Mart., El Oro, Mexico, *J. blancoi* Mart. var. *huehuentensis* R. P. Adams et al., Cerro Huehuento, Durango, Mexico and *J. blancoi* var. *mucronata* (R. P. Adams) Farjon, w. of Maicoba, Chihuahua/ Sonora border, Mexico were analyzed and compared. The volatile leaf oils of *J. blancoi* and *J. scopulorum* were reported in Adams et al. (2006) and Adams (2011), respectively.

## MATERIALS AND METHODS

Specimens used in this study were: *J. blancoi*: Adams 6849-6851 & 6903-6904, 2580 m, 7 km s of Carmona (s of El Oro) Mexico, Mexico; *J. blancoi* var. *huehuentensis* Adams 10247-10251, 3227 m, Cerro Huehuento, Durango, Mexico; *J. blancoi* var. *mucronata*, Adams 8453-8463, 1180 m, 19 km w of Maicoba, Chihuahua/ Sonora border, Mexico; *J. scopulorum*, Adams 2010-2024, 2012 m, Durango, CO, USA; putative *J. scopulorum*, Adams 2501-2510, 2120 m, Colonia Pacheco, Chihuahua, Mexico. In addition, DNA was extracted from a 1978 herbarium specimen (Adams 2512, Colonia Pacheco, Mexico). Voucher specimens are deposited at BAYLU, Baylor University.

Isolation of Oils - See Adams (2011). Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Terpenoids Data Analysis - Terpenoids (as per cent total oil) were coded and Gower or Manhattan metric (Adams, 1975; Gower, 1971) were computed among all populations using equal character weighting. Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

DNA was extracted from the Adams 2512 herbarium specimen (1978) by use of a Qiagen mini-plant kit as per manufacturer's instructions. Degraded DNA was obtained that ranged from 2600 - 100 bp (mode 300 bp).

ITS (nrDNA) amplification was performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E or K (final concentration: 50 mM KCl, 50 mM Tris-HCl [pH 8.3], 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl<sub>2</sub>, according to the buffer used) 1.8 µM each primer (see Adams, Bartel and Price, [2009] for buffer enhancers used).

Primers (5'-3'):

ITS: ITSA-42F GAT TGA ATG ATC CGG TGA AGT  
ITSB+57R ATT TTC ATG CTG GGC TCT

However, the sequences were messy after about 350- 400 bp. Additional internal primers were sequenced by 'walking' along sequenced data:

ITS463F CTG TGT TAA GGA TGG GTG CA Tm 59.6°C  
ITS650F GCG CAC CTT AGA AAT CCA Tm 57.4°C  
ITS739F AAC GGA TAT CTC GGC TCT, Tm 52°C

The PCR reactions were purified by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and cleaned using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/>). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2006).

## RESULTS AND DISCUSSION

The oils are of these taxa are very similar (Table 1). The oils from Colonia Pacheco and *J. scopulorum* share three unique components p-cymen-8-ol, methyl eugenol and safrole (Table 1). Colonia Pacheco (CM) uniquely shares one component with all the varieties of *J. blancoi*: hexanoic acid, 4-methyl, methyl ester. Colonia Pacheco shares two components with one or more *J. blancoi* varieties (but not *J. scopulorum*): 2-heptyl acetate and myrtenol.



Principal Coordinates Ordination (PCO) reveals the overall similarities in the oils (Figure 1). The minimum spanning network connects the Colonia Pacheco (CM, table 1) to *J. scopulorum* from Durango, CO (0.656). However, *J. scopulorum* (Durango, CO) is about as similar to *J. blancoi* var. *huehuentensis* (0.646). The oil from the Colonia Pacheco juniper is much less similar to *J. b.* var. *huehuentensis*, the next most similar node (dashed line, Fig. 1).

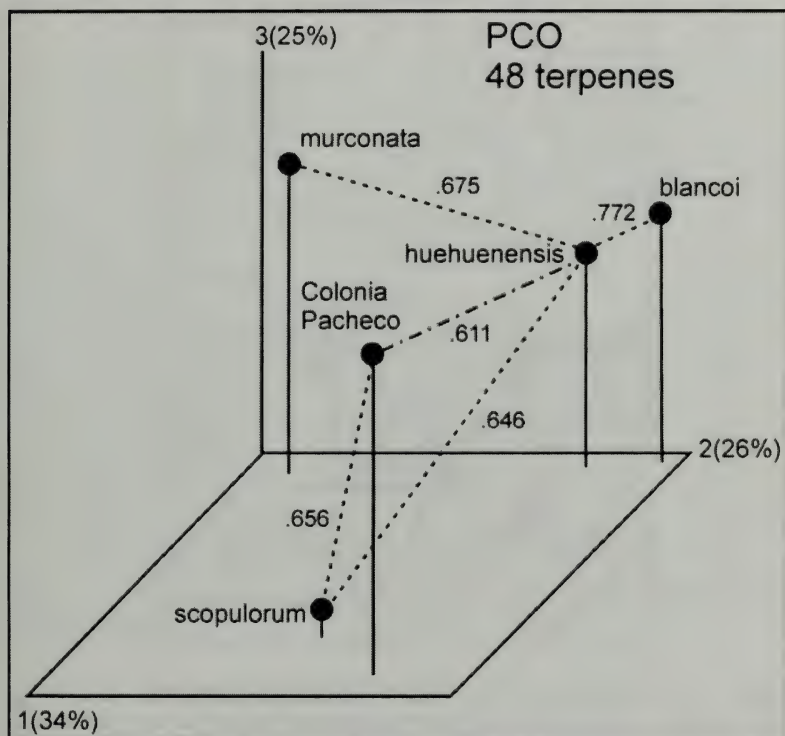


Figure 1. PCO based on 48 terpenes with a minimum spanning network superimposed (dotted line). The dashed line shows the second nearest link from Colonia Pacheco to *J. blancoi* var. *huehuentensis* (0.611).

Mapping the minimum spanning network (Fig. 2) gives a spatial perspective to the similarities. Notice that *J. b. var. mucronata* is less than 200 km from Colonia Pacheco and that the nearest known population of *J. b. var. huehuentensis* (Cerro Mohinora, Chi.) is about 350 km.

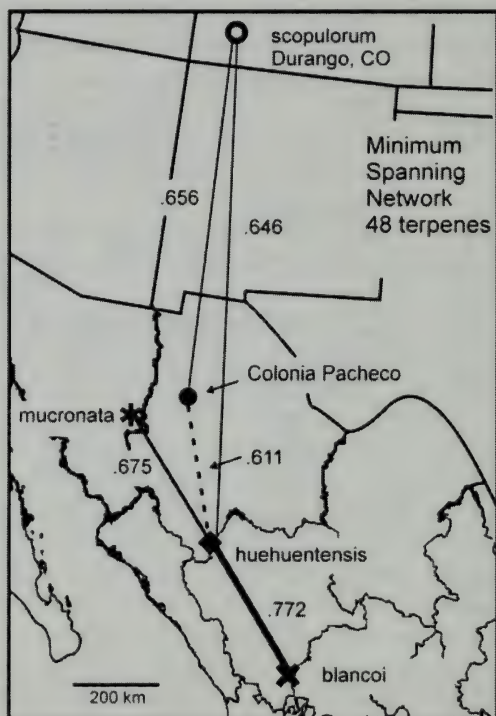


Figure 2. Minimum spanning network based on 48 terpenes. The heavy lines indicate greater similarity. The dashed line is the second nearest link to Colonia Pacheco (0.611 to *J. b. var. huehuentensis*).

However, the oils at Colonia Pacheco are much more similar to *J. scopulorum* (Durango, CO, 0.656) than to *J. b. var. huehuentensis* (0.611, dashed line in Fig. 2).

The most robust analysis of DNA sequences for the smooth-leaf margined junipers of Mexico revealed (Fig. 3) that three species

are resolved: *J. blancoi* (and its varieties), *J. scopulorum* and *J. virginiana* (Adams, 2009).

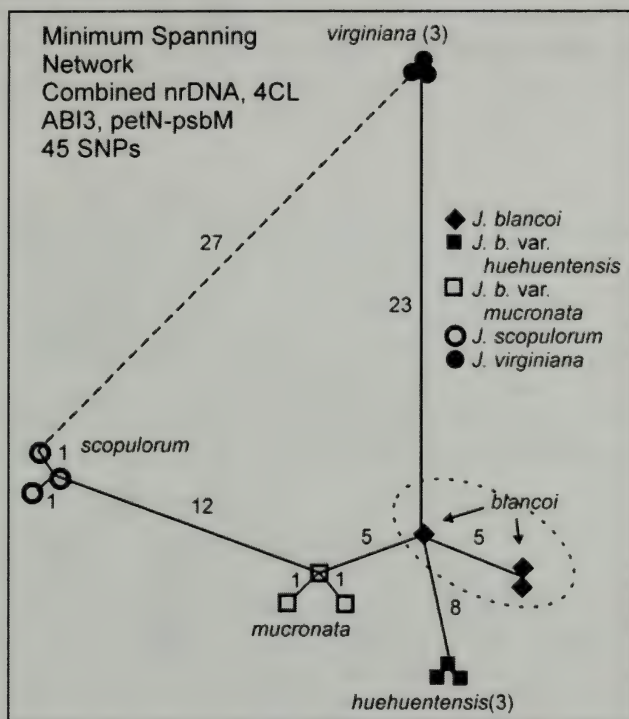


Figure 3. Minimum spanning network based on 45 SNPs (from Adams, 2009).

Sequencing the degraded DNA for nrDNA from the 1978 specimen from Colonia Pacheco proved to be somewhat difficult and additional internal primers were necessary. Analysis of the SNPs (2 single mutations in 2512 were ignored) revealed that the sequence differs by only 1 SNP from *J. blancoi* (El Oro) but differed by 4 SNPs from *J. scopulorum* (Fig. 4, dashed line). The nrDNA data shows





have a few or some two-seeded, bilobed cones on female trees. Therefore, the cone shape is not definitive in classifying the junipers at Colonia Pacheco. The scale leaf-tips of the junipers at Colonia Pacheco are acute as found in *J. blancoi* var. *blancoi* and *J. b.* var. *huehuentensis*. The leaf-tips are not obtuse, as usually found in *J. scopulorum*, nor mucronate as found in *J. b.* var. *mucronata* (Adams, 2008).

Considering the terpenoids, nrDNA, morphology, distribution and ecology, the juniper at Colonia Pacheco seems more likely to be the northern-most population of *J. blancoi* than a relict of *J. scopulorum* from the Pleistocene. But gene flow between *J. blancoi* and *J. scopulorum* seems likely during the Pleistocene, making interpretation of isolated populations difficult. A few specimens of putative *J. scopulorum* have been collected from northwestern Sonora (van Devender, ASU, ARIZ). Analysis of DNA from these specimens (Adams, in progress), may show that typical *J. scopulorum* is present in Mexico.

### ACKNOWLEDGEMENTS

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Table 1. Comparisons of the leaf essential oils for *J. scopulorum* (SC), Durango, CO, USA, putative *J. scopulorum* (CM), Colonia Pacheco, Mexico, *J. blancoi* (BL), *J. blancoi* var.. *huehuentensis* (BH) and *J. blancoi* var. *mucronata* (BM), and. Components that tend to separate the species are highlighted in boldface.

KI	Compound	SC	CM	BM	BH	BL
899	2-ethyl-2-pentanol*	0.1	0.5	0.4	0.3	0.4
926	tricyclene	t	-	t	-	-
931	$\alpha$ -thujene	1.1	1.1	1.5	1.2	1.6
<b>939</b>	<b><math>\alpha</math>-pinene</b>	<b>4.7</b>	<b>1.9</b>	<b>2.2</b>	<b>1.6</b>	<b>1.8</b>
953	$\alpha$ -fenchene	t	t	t	t	t
953	camphene	0.1	0.1	0.1	t	t
976	sabinene	46.3	46.0	52.6	45.5	48.3
980	$\beta$ -pinene	t	t	t	t	t
991	myrcene	1.3	1.7	2.9	2.0	2.4
<b>996</b>	<b>hexanoic acid, 4-methyl, methyl ester*</b>	-	<b>1.1</b>	<b>0.7</b>	<b>0.2</b>	<b>0.2</b>
<b>1001</b>	<b><math>\delta</math>-2-carene</b>	<b>t</b>	<b>0.2</b>	<b>0.4</b>	<b>0.4</b>	<b>0.2</b>
1005	$\alpha$ -phellandrene	0.1	t	0.1	0.2	0.2
1011	$\delta$ -3-carene	0.1	0.1	0.1	-	t
1018	$\alpha$ -terpinene	1.1	0.7	1.2	1.9	1.8
1026	p-cymene	0.5	1.3	0.3	0.2	0.1
<b>1031</b>	<b>limonene</b>	<b>5.6</b>	<b>1.2</b>	<b>1.3</b>	<b>1.1</b>	<b>1.6</b>
1031	$\beta$ -phellandrene	0.8	0.8	1.0	1.1	0.4
1034	2-heptyl acetate	-	0.1	-	-	0.1

KI	Compound	SC	CM	BM	BH	BL
1050	(E)- $\beta$ -ocimene	0.1	0.1	0.5	0.2	0.2
1062	$\gamma$ -terpinene	1.9	1.0	2.0	3.3	2.9
1068	cis-sabinene hydrate	1.4	1.4	1.5	1.0	1.1
<b>1067</b>	<b>cis-linalool oxide (furanoid)</b>	<b>t</b>	-	-	-	-
1088	terpinolene	1.0	0.6	1.1	1.3	1.1
1091	2-nonanone	0.3	2.6	0.9	0.1	1.9
1097	trans-sabinene hydrate	1.0	1.0	1.0	1.0	1.0
1098	linalool	1.3	2.6	1.5	0.1	0.8
1102	n-nonanal	t	t	0.1	0.2	0.3
1114	trans-thujone(= $\beta$ -thujone)	0.1	t	0.1	0.1	t
1121	cis-p-menth-2-en-1-ol	0.4	0.5	0.4	0.5	0.5
1140	trans-p-menth-2-en-1-ol	0.2	0.3	0.2	0.3	0.3
1143	camphor	0.2	-	-	0.1	-
1148	camphene hydrate	t	-	-	-	-
1177	terpinen-4-ol	5.8	6.6	3.9	7.6	6.2
1179	naphthalene	-	-	-	t	-
<b>1183</b>	<b>p-cymen-8-ol</b>	<b>t</b>	<b>0.1</b>	-	-	-
1189	$\alpha$ -terpineol	0.2	0.2	0.2	0.3	0.2
<b>1191</b>	<b>myrtenol</b>	-	<b>0.1</b>	<b>0.1</b>	-	-
1193	cis-piperitol	0.1	t	0.1	0.1	0.2
1196	methyl chavicol	-	0.1	-	-	-



KI	Compound	SC	CM	BM	BH	BL
1205	trans-piperitol	0.1	0.1	0.1	0.2	0.2
1228	citronellol	0.5	0.1	0.1	-	0.1
1252	piperitone	t	0.2	0.2	t	0.1
1252	trans-sabinene hydrate acetate	0.1	-	-	-	-
1257	4Z-decen-1-ol	0.1	0.2	0.2	0.1	0.1
1274	pregeijerene B	6.0	1.2	2.0	3.2	3.2
1285	bornyl acetate	0.7	t	0.2	t	0.2
1285	<b>safole</b>	t	<b>5.6</b>	-	-	-
1287	trans-linalyl oxide (pyranoid)	-	-	0.1	-	-
1291	<b>2-undecanone</b>	t	<b>0.3</b>	<b>0.1</b>	-	-
1401	<b>methyl eugenol</b>	<b>0.2</b>	<b>3.7</b>	-	-	-
1418	(E)-caryophyllene	0.2	0.2	0.6	0.5	t
1442	guaiaiene <6,9->	0.2	0.1	0.1	0.1	0.1
1451	(Z)-methyl iso-eugenol	0.1	-	-	0.1	0.1
1455	$\alpha$ -humulene	-	-	-	t	-
1466	9-epi-(E)-caryophyllene	t	-	-	-	-
1477	$\gamma$ -muurolene	0.1	-	-	t	-
1480	<b>germacrene D</b>	<b>0.1</b>	<b>0.2</b>	<b>0.2</b>	<b>0.3</b>	<b>0.1</b>
1493	epi-cubebol	0.1	t	0.1	0.1	-
1499	$\alpha$ -muurolene	0.1	0.1	0.2	0.2	t
1513	<b><math>\gamma</math>-cadinene</b>	<b>0.2</b>	<b>0.2</b>	<b>0.5</b>	<b>0.3</b>	-

KI	Compound	SC	CM	BM	BH	BL
1524	$\delta$ -cadinene	0.3	0.4	0.9	0.9	t
1535	$\alpha$ -copaen-11-ol	0.3	t	0.1	0.1	0.1
1538	$\alpha$ -cadinene	t	t	0.2	0.1	t
1549	elemol	4.3	2.1	2.2	4.0	2.5
1555	elemicin	-	0.5	-	-	-
1561	germacrene B	0.2	t	-	0.3	0.2
1564	(E)-nerolidol	-	-	-	t	-
1574	germacrene D-4-ol	0.8	1.0	2.6	0.9	0.1
1606	$\beta$ -oplopenone	0.2	0.3	0.5	0.4	-
1630	$\gamma$ -eudesmol	0.3	0.3	0.1	0.6	0.2
1640	epi- $\alpha$ -cadinol	0.5	0.3	0.5	0.5	0.2
1640	epi- $\alpha$ -muurolol	0.4	0.2	0.4	0.5	0.1
1645	$\alpha$ -muurolol (=torreyol)	t	t	0.1	t	-
1649	$\beta$ -eudesmol	0.4	0.2	0.2	0.8	0.3
1652	$\alpha$ -eudesmol	0.6	0.4	0.7	1.1	0.3
1653	$\alpha$ -cadinol	0.5	0.4	0.7	0.8	0.2
1666	bulnesol	0.2	0.1	0.2	0.3	0.2
1689	shyobunol	-	-	-	-	0.2
1701	cis-thujopsenol	-	t	-	-	-
1739	oplopanone	-	t	-	-	-
1762	8- $\alpha$ -acetoxylemo, isomer	0.1	0.1	0.1	0.2	0.2

KI	Compound	SC	CM	BM	BH	BL
1789	8- $\alpha$ -acetoxylemol	5.9	2.9	2.8	5.3	4.2
<b>2055</b>	<b>manool</b>	<b>t</b>	<b>0.6</b>	<b>1.1</b>	<b>1.8</b>	<b>7.6</b>
2087	abietadiene	t	t	0.1	0.2	t
<b>2135</b>	<b>diterpene, 41,69,255,298</b>	<b>0.5</b>	<b>0.5</b>	<b>t</b>	<b>0.4</b>	<b>t</b>
<b>2283</b>	<b>sempervinol</b>	<b>t</b>	<b>t</b>	<b>0.4</b>	<b>0.2</b>	<b>0.5</b>
<b>2298</b>	<b>4-epi-abietal</b>	<b>0.1</b>	<b>0.1</b>	<b>0.5</b>	<b>0.5</b>	<b>0.4</b>
<b>2314</b>	<b>trans-totarol</b>	<b>t</b>	<b>t</b>	<b>0.2</b>	<b>0.1</b>	<b>0.2</b>
2331	trans-ferruginol	-	-	t	t	t

KI = Kovat's Index on DB-5(=SE54) column. \*Tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

## DNA BARCODING A JUNIPER: THE CASE OF THE SOUTH TEXAS DUVAL COUNTY JUNIPER AND SERRATE JUNIPERS OF NORTH AMERICA

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### ABSTRACT

The utilization of 4351 bp from five gene regions (nrDNA, petN-psbM, trnD-trnT, trnL-trnF, trnS-trnG) was sufficient to accurately identify an unknown juniper species from Duval County, TX as *J. pinchotii*. Bayesian, Maximum Likelihood, Parsimony, and NJ analyses were equally adept in identifying the juniper, but UPGMA was barely able to identify it and Minimum Linkage was equivocal. A robust phylogeny of the serrate-leaf junipers of North America is presented as a consequence of the study. *Phytologia* 93(2): 146-154 (August 1, 2011).

**KEY WORDS:** *Juniperus pinchotii*, serrate-leaf junipers, North America, barcode, phylogeny, Cupressaceae, nrDNA, petN-psbM, trnD-trnT, trnL-trnF, trnS-trnG.

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Recently, a colleague, William Carr, while doing routine collecting in Duval County, Texas, obtained samples from a small juniper shrub growing on Cedro Hill on white claystone or caliche of the Fant Tuff member of the Catahoula and Frio formations. There are about 15 juniper plants in a 15 x 20 m area, growing generally with or under the shrubs *Gochnatia hypoleuca* and *Leucophyllum frutescens* in very xeric conditions. The juniper specimen had ball-like foliage without terminal whips (due to drought) and one very small, immature



seed cone that had (apparently) turned blue during drying. Without terminal whip leaves and mature seed cones, most *Juniperus* are impossible to identify to the species level. The unknown juniper had serrate-leaf margins, which put it in the serrate *Juniperus* group of North America; but that group contains 21 species (Adams, 2011) and since the Duval Co. site is several hundred miles from the nearest known, natural population of serrate *Juniperus* routine identification proved to be impossible.

There has recently been considerable discussion about using DNA barcoding to identify plants (Chase and Fay, 2009; Seberg and Petersen, 2009; Chase et al. 2007; Cowan et al. 2006; Newmaster et al. 2006); Kress et al. 2005). However, Seberg and Petersen (2009) found that even using 6 average sized barcode regions would not identify all of the 86 known *Crocus* species.

Because several recent DNA sequencing studies of serrate-leaf *Juniperus* have been published (Mao et al. 2010; Willson et al. 2008; Adams and Kauffmann, 2010; Adams, 2009; Adams, Schwarzbach and Morris, 2010), it seemed an opportune time to complete sequences for all the serrate junipers of North America for nrDNA, petN-psbM, trnD-trnT, trnL-trnF and trnS-trnG and test if these five regions would be sufficient to identify the unknown Duval County juniper.

## MATERIALS AND METHODS

Plant material: *J. pinchotii*, *W. Carr* 28809 (Adams 12248 in lab), 27° 45' 30.8"N, 98° 41' 58.5"W, 666ft., Cedro Hill, Duval County, Texas 26 Apr, 2010 and Adams 12534-12538, same location, 13 Nov. 2010. Voucher specimens are deposited at Baylor University (BAYLU) and the University of Texas (TEX).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

*PCR amplification* Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq

polymerase, 15  $\mu$ l 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200  $\mu$ M each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM  $MgCl_2$  according to the buffer used) 1.8  $\mu$ M each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (QIAGEN, Valencia, CA). The gel purified DNA band with the appropriate primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Sequence datasets were analyzed using Geneious v. 5.1 (Drummond et al. 2010), the MAFFT alignment program (Kato et al. 2005), and the PAUP\* program, version 4.0b10 (Swofford 2003) for neighbor joining, parsimony, and maximum likelihood tree searches. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion. Sequences were aligned by use of MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/>). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009, Adams, 1975). GenBank sequences were downloaded as available to complement the sequences for all five gene regions for all of the 23 taxa (2 accessions each) of serrate junipers present in N. America, plus the unknown Duval County juniper and two accessions of *J. virginiana*, Tennessee (as an entire-leaf juniper outgroup).

## RESULTS AND DISCUSSION

The concatenated data set was composed of 4,351 bp from nrDNA, petN-psbM, trnD-trnT, trnL-trnF and trnS-trnG sequences. Bayesian analysis (Fig. 1) placed the Duval juniper in a clade with *J. pinchotii*. All of the serrate junipers are well resolved. However, the position of *J. californica* seems odd as it is normally considered a sibling species to the western junipers (*J. grandis*, *J. occidentalis* and *J.*

*osteosperma*). It appears that some major mutations have occurred in the nrDNA of *J. californica* and this perhaps explains its unusual position. Analyses of the data set without nrDNA resulted in an unresolved clade containing *J. angosturana*, *J. pinchotii* and the Duval juniper as well as several other unresolved taxa (data not shown).

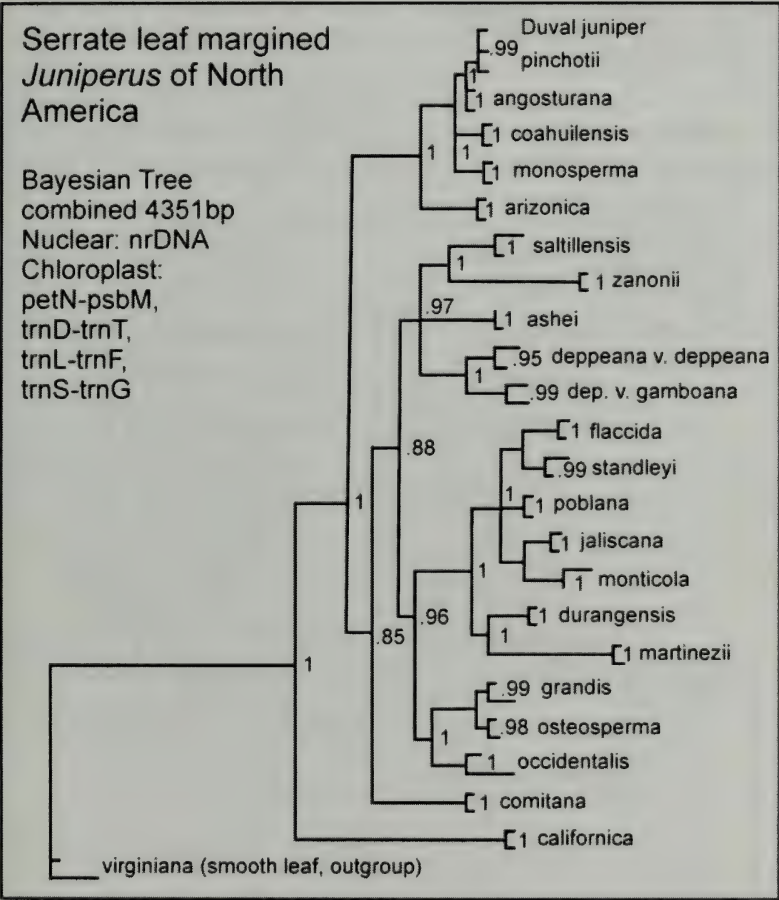


Figure 1. Bayesian tree of the serrate *Juniperus* of North America with the Duval juniper in a clade with *J. pinchotii*. Numbers at the branch points are posterior probabilities (0 - 1).

A NJ tree was very similar to the Bayesian tree. The Duval juniper was placed in a clade with *J. pinchotii* (Fig. 2). In fact, except for differences in the relationships of the four major clades, Bayesian and NJ

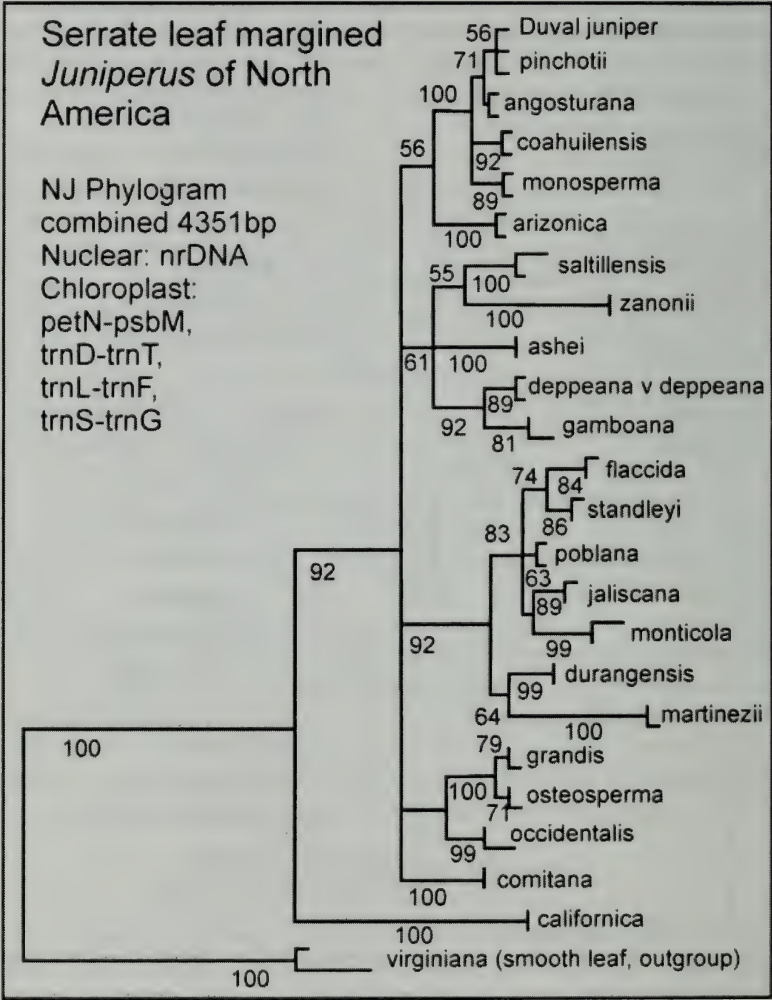


Figure 2. NJ tree of the serrate *Juniperus* of North America with the Duval juniper in a clade with *J. pinchotii*. Numbers at the branch points are bootstrap values as percent (1000 reps.).



trees were nearly identical (Fig. 1 vs. 2). In both cases, the Duval juniper was placed in a clade with *J. pinchotii*.

In addition to Bayesian and NJ analyses, Maximum Likelihood, Parsimony, UPGMA and Minimum Linkage analyses were computed. Interestingly, Bayesian, Maximum Likelihood and Parsimony gave exactly the same clade topography for the *J. pinchotii*, *J. angosturana*, *J. coahuilensis*, *J. monosperma* clade (Figure 3). The UPGMA diagram was intermediate between Bayesian and NJ and barely placed the Duval juniper with *J. pinchotii* (Figure 3). The Minimum Linkage diagram was slightly different and was inconclusive in classifying the Duval juniper (Figure 3).

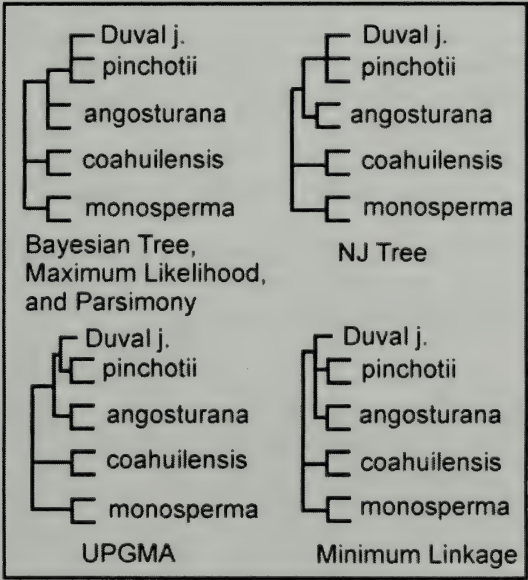


Figure 3. Comparison of six methods of data analyses.

**The rest of the story** - On 13 Nov. 2010, the senior author visited Cedro Hill and found approximately 15 juniper shrubs, of which one had 7 mature seed cones and long terminal whips (due to copious rainfall in the spring and summer, 2010. The seed cones were copper-red, as found only in *J. pinchotii* in the western hemisphere. The terminal whip leaves

had ruptured glands with a white exudate, typical of *J. pinchotii*. The plant was easy to identify as *J. pinchotii*. So after spending months of lab work and thousands of dollars, the mystery was easy to solve by examining the plant at the proper time. Perhaps we should continue to teach students classical taxonomy!

The disjunct Duval Co. population is shown on the distribution map for *J. pinchotii* (Fig. 4) and appears to be about equidistant from the central populations in Texas and the disjunct population in Mexico on the Coahuila - Nuevo Leon border.

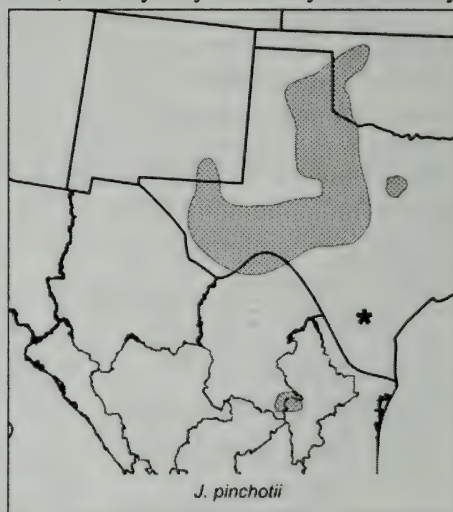


Figure 4. Distribution of *J. pinchotii*. The star represents the Duval Co. population.



Figure 5. *Juniperus pinchotii* on Cedro Hill, Duval Co., Texas.

## ACKNOWLEDGEMENTS

Special thanks to Bill Carr for providing the specimen and detailed information about the site. Thanks to David Kitner, Foreman, Duval County Ranch, Freer, TX for providing access and arranging a trip to Cedrol Hill. This research was supported in part with funds from Baylor University. Thanks to Tonya Yanke for lab assistance.

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***Jaltomata atiquipa***  
**southern Peru**



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Contents

W. H. Blackwell. The genus *Lagena* (Stramenophila: Oomycota), Taxonomic history and nomenclature.....157

R. P. Adams. *Juniperus virginiana* in the Serranias del Burro Mountains, Coahuila, Mexico: A Pleistocene Relict.....168

B. L. Turner. Biological status of *Hedeoma drummondii*, *H. reverchonii* (Lamiaceae) and closely related taxa.....174

B. L. Turner. Systematic study of the *Dalea nana* complex (Fabaceae) .....181

R. P. Adams, J. Murata, H. Takahashi and A. E. Schwarzbach. Taxonomy and evolution of *Juniperus communis*: Insight from DNA sequencing and SNPs.....185

R. P. Adams and J. Poole. Low variability of DNA fingerprints of Texas snowbells: Conservation implications.....198

T. Mione, S. Leiva G., L. Yacher and A. M. Cameron. *Jaltomata atiquipa* (Solanaceae): A new species from southern Peru.....203

R. P. Adams, R. M. Lanner, M. Kauffmann and D. Thornburg. Taxonomy of infraspecific taxa of *Abies concolor*: leaf essential oils of var. *concolor* and var. *lowiana* - Errata.....208

R. P. Adams, R. M. Lanner, M. Kauffmann and D. Thornburg. Taxonomy of infraspecific taxa of *Abies concolor* based on DNA sequences from nrDNA and four chloroplast regions.....221

D. B. Ward. Keys to the flora of Florida - 28, *Iris* (Iridaceae).....231

Cover Photo. *Jaltomata atiquipa* photo by Tom Mione, see p. 203.

✓ B. L. Turner. A new species of <i>Scutellaria</i> (Lamiaceae) from Oaxaca, Mexico.....	241
R. P. Adams. Geographic variation in the leaf essential oils of <i>Juniperus californica</i> .....	245
B. L. Turner and M. Martinez. Systematic reassessment of the North American <i>Physalis viscosa</i> complex (Solanaceae).....	260
✓ B. A. Sorrie. Transfer of North American <i>Helianthemum</i> to <i>Crocianthemum</i> (Cistaceae): New combinations.....	270
Editorial Note: Back issues of Phytologia Memoirs.....	272

## THE GENUS *LAGENA* (STRAMENOPILA: OOMYCOTA), TAXONOMIC HISTORY AND NOMENCLATURE

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### ABSTRACT

A significant and morphologically distinct plant parasite, affecting roots of cultivated wheat and related grasses, *Lagena* has received little taxonomic attention. This genus, of oomycete affinity, has proved difficult to place with certainty in any particular oomycete group, especially in the absence of molecular sequence data. The generic nomenclature of *Lagena* was also uncertain, given a proposed replacement name, *Lagenocystis*, which I argue represents an unnecessary nomenclatural change. Several taxa (or potential taxa) have been, directly or indirectly, suggested for inclusion in *Lagena*. However, available evidence suggests retention of *Lagena* as monotypic, the only certain species being *L. radiculicola*. *Phytologia* 93(2): 157-167 (August 1, 2011)

**KEY WORDS:** barley, gametangia, hosts, *Lagenidium*, *Lagenocystis*, oospore, parasite, *Pythium*, roots, rye, sporangia, wheat, zoospores.

---

Vanterpool and Ledingham (1930) described the genus *Lagena* for a “phycomycete” (oomycete) parasite found in wheat, rye and barley roots in fields in Saskatchewan. A single species, *L. radiculicola*, was included in this genus. One to several thalli may occur in individual host cells, such as root-hair or cortical cells. The simple, sac-like thallus (Fig. 1) usually remains connected to the host cell by a persistent neck and “collar” (determined by Barr and Désaulniers, 1990, to be contributed by host callus material in response to penetration by the parasite). The thallus becomes multinucleate, and is holocarpic, yielding infective zoospores (Figs. 1, 5, 7) formed in an external vesicle at the tip of a discharge tube (Figs. 3, 4). Empty sporangia may be evident (Fig. 6), still exhibiting the neck region and discharge tube. Undifferentiated gametangia (resembling sporangia), bridged by

conjugation tubes, were described in (apparently isogamous) sexual reproduction leading to oospore formation (Figs. 8-10), cf. Vanterpool and Ledingham (1930). Additional observations by Truscott (1933), in Ontario, and Macfarlane (1970), in Europe, further established morphological variation of this unique organism. Truscott noted that the thallus (sporangium) may be branched (Fig. 2). Macfarlane observed that the thallus could assume a more elongate, tubular form, perhaps becoming twisted, but still not growing from one host cell to another. Photographs of oospores (resting spores) of *L. radicola* by Macfarlane (1970) show that these can be eccentric [a term related to the position of the reserve food globule in the oospore, cf. Dick, 1969] compared to the centric spores illustrated by Vanterpool and Ledingham (1930)—cf. Figs. 11, 12. Vanterpool and Ledingham illustrated development of a single oospore in a female gametangium; however, Truscott (1933) thought several oospores might develop in one such structure. The occasional abundance of *Lagena radicola*, its association with species of *Pythium* in “browning root rot” of cereals, and infection of related wild grasses (e.g., *Agropyron repens*) were also observed. Host symptoms involved the stunting, tip-crooking, and necrosis of small infected lateral roots. *Lagena* was included by Sprague (1950) in *Diseases of Cereals and Grasses in North America*.

### SYSTEMATIC RELATIONSHIPS

The possible taxonomic relationship of *Lagena* with *Lagenidium* and *Pythium* was suggested by Vanterpool and Ledingham (1930), a conclusion generally supported by others (Fitzpatrick, 1930; Bessey, 1950; Macfarlane, 1970). Sparrow (1939) noted resemblance of *Lagena* to single-celled species of *Lagenidium* infecting algae or rotifer eggs; see also Seymour and Johnson (1973). Sparrow (1960) placed *Lagena* in the Lagenidiaceae, dealing at the time only cursorily with the genus since it is not aquatic. Karling (1981) noted that Vanterpool and Ledingham (1930) considered *Lagena* to possibly represent a link between the Lagenidiaceae and Pythiaceae. Karling (1981) believed *Lagena* to have characteristics in common with *Lagenidium* and *Myzocyttium* (another genus in the Lagenidiaceae), and with *Pythium*.

The systematic position of *Lagena* continued to be uncertain. Dick (1971) thought that oospore structure of *Lagena* (“*Lagenocystis*”)



was similar to certain oospores of the Saprolegniaceae (see Dick, 1969, re: oomycete oospores). Seymour and Johnson (1973), however, believed that some characteristics of *Lagena* oospores were suggestive of oospores of the Leptomitaceae (a family related to the Saprolegniaceae). Seymour and Johnson (1973) also mentioned the potential relationship of an unnamed (*Lagenidium*-like) rotifer parasite they described—and, by associative discussion, of *Lagena radiculicola*—to *Myzocyttium*, a genus distinguished from *Lagenidium* by comparatively undifferentiated sexual structures. In contrast to *Lagena* (and *Lagenidium*), gametangial copulation in *Myzocyttium* is said to often be poroidal, i.e., not necessarily involving obvious fertilization tubes (cf. Canter, 1947; Karling, 1981). Although male and female gametangia of *Myzocyttium* are often more similar than in *Lagenidium*, they are often more readily distinguished than in *Lagena*. Barr and Désaulniers (1990) illustrated variation in number and shape of resting spores (oospores) of *L. radiculicola* consistent with collective observations of earlier investigators (Vanterpool and Ledingham, 1930; Truscott, 1933; Macfarlane, 1970). Barr and Désaulniers (1990) posited a general lagenidialean affinity for *Lagena*, not inconsistent with views, for example, of Vanterpool and Ledingham (1930) and Sparrow (1960).

The zoospore of *Lagena radiculicola* has been discussed as laterally biflagellate (cf. Karling, 1981) and subapically biflagellate (cf. Barr and Désaulniers, 1987). Although apparently variable in this regard (cf. Dick, 2001), these zoospores (Figs. 5, 7) are always biflagellate, and the two flagella (often oriented in at least somewhat different directions) are never truly apical—features suggestive of general stramenopilous affinity. Barr and Désaulniers (1987) described the structure and ultrastructure of the *Lagena* zoospore as indicating probable relationship to Oomycetes; however, they could not pin down this connection further, since the zoospore of *Lagena* lacks the concertina-like helix structure in the flagellar transition zone, characteristic of most oomycete zoospores. A transitional helix, if often simpler in form, is a feature of zoospores of various heterokonts. As noted by Barr and Désaulniers (1987), the *Lagena* zoospore was similar in the absence of a transitional helix to zoospores of the Phaeophyceae. Zoospores of *Lagena* also lack K-bodies or comparable structures (Barr and Désaulniers, 1987), features useful as phylogenetic markers (Powell et al., 1985; Beakes, 1989). The absence of K-body (or similar)

vesicles in the *Lagen*a zoospore is not supportive of a close relationship with members of the Saprolegniaceae and Leptomitaceae (cf. paragraph above), the zoospores of which typically possess such organelles (Beakes, 1989; Powell and Blackwell, 1995). Barr and Désaulniers (1987) could not clearly match the zoospore of *Lagen*a to either the primary or secondary types of zoospores found among Oomycetes (cf. possible evolution of oomycete zoospore types, Blackwell and Powell, 2000). Barr and Désaulniers (1987) did not consider such an attempted categorization as necessarily meaningful for organisms such as *Lagen*a, which produce only one form of zoospore. In a later publication, Barr and Désaulniers (1990), based on various life cycle features, seemed certain that *Lagen*a should be placed in the Oomycetes; beyond that, as mentioned, they merely postulated a lagenidialean connection for *Lagen*a, indicating that "its phylogenetic relationship to other Oomycetes remains unclear." Dick (2001) likewise was unable to determine precise relationships for *Lagen*a, concluding that it was possibly distinct among Peronosporomycetes (Oomycetes), and basing a new family, Lagenaceae (*incertae sedis*), upon it. Within Oomycetes the relationships of *Lagen*a remain uncertain, a circumstance pertaining as well to a number of other holocarpic oomycete genera (see, for example, Blackwell, 2010). Systematic clarification will no doubt occur if molecular sequences for such organisms become available.

## QUESTIONS OF GENERIC NOMENCLATURE

Given (1) the detailed generic description of *Lagen*a by Vanterpool and Ledingham (1930), (2) no real confusion with other oomycete genera, (3) an initial inclusion of only a single species (*L. radicola*), and (4) no obviously included additional species, it might be assumed that there would be little problem with the nomenclature of *Lagen*a. However, a competing generic name, *Lagenocystis* Copeland (1956), was evident in my investigation. *Index Fungorum* indicated *Lagenocystis* as the current generic name for *L. radicola*; whereas, *Index Nominum Genericorum* listed *Lagen*a as correct. Most authors (e.g., Sparrow, 1960; Macfarlane, 1970; Karling, 1981) have used the name *Lagen*a, but others (e.g., Dick, 1971, not 2001) employed *Lagenocystis*. Seymour and Johnson (1973) made reference to *Lagen*a, but observed that Copeland (1956) renamed this organism *Lagenocystis*—a *nomen novum* (cf. ICBN, 2006, Article 7.3) for

*Lagena* Vanterpool & Ledingham (1930). Copeland (1956) provided this replacement name (*Lagenocystis*)—for *Lagena* Vanterpool & Ledingham (1930)—because of the existence of *Lagena* Parker & Jones (1859), a putative earlier homonym (for a different kind of organism).

Since *Lagena* Vanterpool & Ledingham (1930) is not a conserved name (ICBN, 2006), it might at first appear that Copeland (1956) was correct in assigning priority to *Lagena* Parker & Jones (1859), and renaming *Lagena* Vanterpool & Ledingham, as *Lagenocystis*. However, Copeland's action missed the mark on two points. First, in "*Lagena* Parker & Jones," Copeland was referencing the name of a genus of Foraminifera (treated nomenclaturally as an animal). Since the botanical code of nomenclature (ICBN, cf. Principle I) is essentially independent of the zoological code, it is permissible for a plant (or fungus, or oomycete) to bear the same name as an animal. There are a number of instances of this, e.g., *Pieris*, the name of member of the plant family Ericaceae, and of a butterfly. Second, Copeland was incorrect in citing Parker and Jones (1859) as the original source of the name *Lagena* (Foraminifera); this foraminiferan name was first established by Walker and Jacob (1798)—see Cushman (1940), and Loeblich and Tappan (1988). The proper origin of the foraminiferal name, *Lagena*, i.e., by Walker and Jacob (1798), is noted in *Index Nominum Genericorum*. In partial defense of Copeland (1956), it can be mentioned that Parker and Jones (1859) were instrumental in establishing a type for the foraminiferan name *Lagena* (cf. Patterson and Richardson, 1988). In any event, *Lagena* Vanterpool & Ledingham (1930) may be used as the generic name for the oomycete root parasite, regardless of use of *Lagena* as an animal name; there is no need for conservation, or for a substitute name. The name *Lagenocystis* Copeland (1956) should be regarded as superfluous (ICBN, Article 52), and a synonym of *Lagena* Vanterpool & Ledingham (1930), cf. Karling (1981). As it turns out, *Lagenocystis* is also the name of an animal (presumed genus of digenetic trematodes) and of an oomycete—use as an animal name (Yamaguti, 1970) coming after the "fungal" usage.



## ADDITIONAL HOSTS, POSSIBLE ADDITIONAL TAXA

In addition to wheat, *Triticum aestivum*; rye, *Secale cereale*; barley, *Hordeum vulgare*; and relatives (e.g., *Agropyron* sp.), other hosts for *Lagenia radiculicola* have been reported. Truscott (1933) noted finding this parasite in maize and “a number of...wild grasses.” Initially, Vanterpool and Ledingham (1930) had reported occasional attack of finer roots of *Zea mays* under experimental conditions. Macfarlane (1970) noted finding *L. radiculicola* in *Nicotiana debneyi*, and successful inoculation of the parasite in tobacco and cabbage (but not tomato). Macfarlane referred to the finding of an unidentified organism resembling *L. radiculicola* in roots of sugar cane, in Mauritius, by Antoine and Ricaud (1966)—suggesting range extension of this parasite into tropical environments, and raising questions as to host specificity. Karling (1981) mentioned sugar cane (re: the Mauritius “fungus”), along with corn, as a potential host for *L. radiculicola*.

No additional taxa have been added with certainty to *Lagenia*; *L. radiculicola* is still the only generally accepted species. Nonetheless, other possible taxa have been mentioned. Truscott (1933) wondered if branched-thallus individuals (Fig. 2) of *L. radiculicola* might be a taxon distinct from simple, sac-like thallus specimens (Fig. 1), but seemed to dismiss this idea after observing intergrading forms. Sparrow (1939) described a one-celled, saccate, sometimes lobed parasite of eggs and embryos of rotifers, that he named *Lagenidium oophilum*. Sparrow discussed the similarity of the thallus of this rotifer parasite to that of the parasite of wheat roots, *Lagenia radiculicola*. Although Sparrow described his organism (including Latin diagnosis) as a species of *Lagenidium*, he suggested that it could eventually be placed in *Lagenia*, and, if so, that the name would be *Lagenia oophila*. Sparrow thus, intentionally or not, introduced the binomial, *Lagenia oophila*. Sparrow (1960), though, continued to recognize this rotifer parasite as a species of *Lagenidium*, as did Karling (1981). The eventual disposition of Sparrow's *Lagenidium oophilum* remains in question. Dick (1997) included this species under *Myzocytiopsis* as a “doubtful” taxon. *Myzocytiopsis* was established (Dick, 1997) for *Lagenidium*- or *Myzocytiium*-like organisms, with intrasporangial zoosporogenesis, which are parasites of animals (rotifers, aschelminths)—see Pereira and Vélez (2004). Dick (2001) continued to list *M. oophila* (Sparrow) Dick



as a doubtful species of *Myzocytiopsis*. It at least seems clear from the foregoing that *Lagenidium oophilum* Sparrow (1939) has not been accepted as a member of the genus *Lagena*. Seymour and Johnson (1973) described a somewhat similar, unnamed rotifer-egg parasite they likened in some ways to *Lagena radiculicola*; however, zoosporogenesis of their organism is intrasporangial, and there is similarity in other regards as well to *Myzocytiopsis fijiensis* (cf. Seymour and Johnson, 1973; Dick, 1997). Without molecular sequence data, however, any such relationships are difficult to decipher with certainty.

As for other possible taxa of *Lagena*, Dick (1971) briefly discussed an unnamed organism parasitizing the water mold, *Aphanomyces*. He described this parasite as “holocarpic” and “polyoosporous,” with a “reserve globule disposition similar to *Lagenocystis* [*Lagena*] and the eccentric *Achlya* species.” In an earlier reference Dick (1970) noted that oogonia of the *Aphanomyces* invader “contained several apparently eccentric oospores”—suggestive of *Lagena* oospores illustrated by Macfarlane (1970). The *Aphanomyces* parasite, however, was not illustrated, nor was information provided on its thallus morphology or zoospores. Hence, information is too sketchy to assess its taxonomic identity. It was not mentioned in Dick (2001).

A final organism to discuss is associated with leaf-spot disease of *Panicum repens* in India. This grass could be found in dry conditions at certain times of the year and semi-aquatic conditions at others, depending on rainfall. The infecting “fungus” proved to be an oomycete, described as *Petersenia panicicola* by Thirumalachar and Lacy (1951). The genus *Petersenia* was established by Sparrow (1934) for *Olpidiopsis*-like organisms with lobed thalli, parasitic in certain marine algae, or water-molds (see also Sparrow, 1960). The existence of a “terrestrial” *Petersenia* was unusual, and Thirumalachar and Lacy (1951) thought the thickened “resting sporangia” of *P. panicicola* to represent a land-existence adaptation. Perhaps in consideration of its generally terrestrial habitat, Dick (2001) listed *P. panicicola* (as a “doubtful” but not excluded species) under *Lagena*. Under *Petersenia*, Dick (2001) listed *P. panicicola* as “excluded” and “possibly related to *Lagena*.” Information and illustrations in Thirumalachar and Lacy (1951) and Karling (1981), though, would lead one to question Dick’s tentative placement in *Lagena*. Sporangia of *Petersenia panicicola* are

often deeply and irregularly to more or less regularly lobed, the persistent lobing in some cases being almost “stellate” in appearance. Although there is variation in thallus/sporangial lobing of *Lagena radicola*, it does not attain the strikingly and firmly lobed appearance of *Petersenia panicicola*. Also, zoosporogenesis in *Petersenia panicicola* is apparently intrasporangial (based on sparse information in Thirumalachar and Lacy), not extrasporangial and vesicular (as in *Lagena*). Thus, *P. panicicola* seems better placed morphologically in *Petersenia* than in *Lagena*; it is, in fact, recognized under *Petersenia* in *Index Fungorum*. *Lagena* should for now remain monotypic.

### ACKNOWLEDGMENTS

I thank Dr. W. Wallace Martin, Randolph-Macon College, Virginia; and Dr. Gordon W. Beakes, Newcastle University, UK, for thoughtful review of this manuscript. I also thank Dr. Martha J. Powell, University of Alabama, for helpful suggestions.

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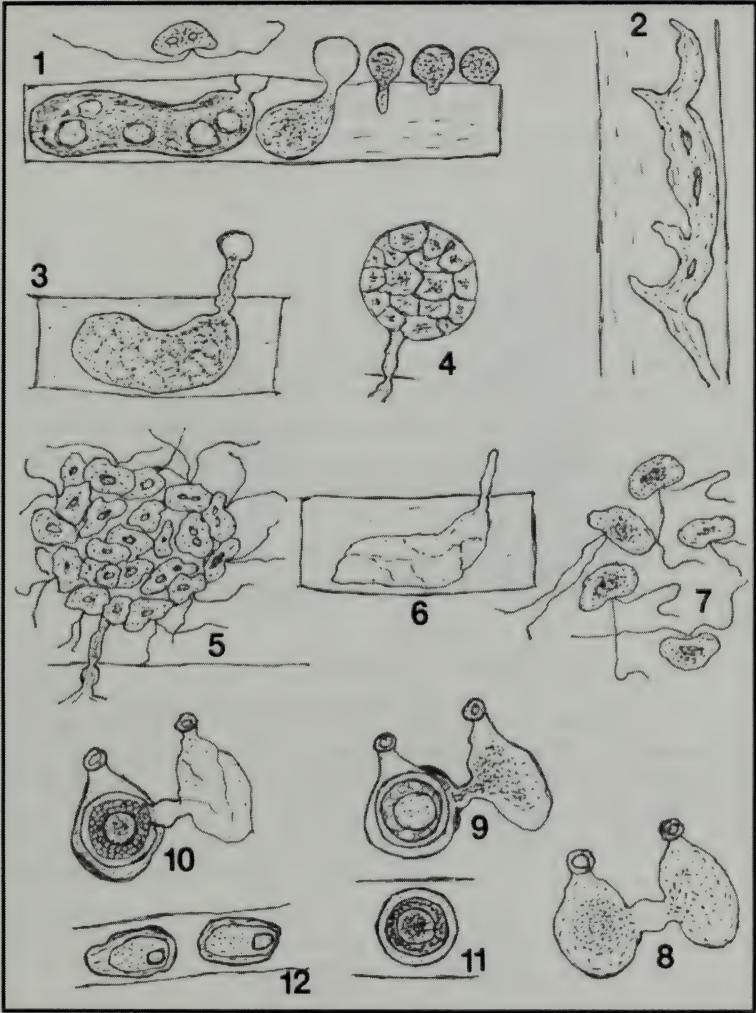
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Figs. 1-12: *Lagena radiculicola* (infecting wheat and barley). Fig. 1: Sac-like thallus developed from encysted zoospore; neck and collar area of thallus are attached to the host cell. Fig. 2: Branched thallus. Fig. 3: Sporangium; discharge tube forms from neck area. Fig. 4: Zoospores cleaved in vesicle at tip of discharge tube. Fig. 5: Mass of zoospores. Fig. 6: Empty sporangium after discharge. Fig. 7: Free-swimming, biflagellate zoospores. Fig. 8: Gametangia with conjugation tube. Fig. 9: Contents of male gametangium flowing toward oosphere in female gametangium. Fig. 10: Oospore developing, post fertilization. Fig. 11: Mature, centric oospore. Fig. 12: Eccentric form of oospore. Figs. 1,3,4,5,6,7,8,9,10,11 after Vanterpool and Ledingham, 1930; Fig. 2 after Truscott, 1933; Fig. 12 after Macfarlane, 1970.





Figures 1-12. See caption of previous page.

**JUNPERUS VIRGINIANA IN THE SERRANIAS DEL BURRO  
MOUNTAINS, COAHUILA, MEXICO:  
A PLEISTOCENE RELICT**

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**ABSTRACT**

The nrDNA sequence of the smooth leaf margined juniper of the Serranias del Burro was compared with *J. virginiana*, *J. scopulorum* and *J. blancoi*. nrDNA from S. del Burro was most similar to *J. virginiana* and then to the *J. blancoi*/*scopulorum* juniper from Colonia Pacheco. The Serranias del Burro population differs by 3 SNPs in its nrDNA from typical *J. virginiana* (central Texas) and appears to be a Pleistocene relict. *Phytologia* 93(2):168-173 (August 1, 2011).

**KEY WORDS:** *Juniperus virginiana*, *J. scopulorum*, *J. blancoi*, geographic variation, nrDNA, SNPs, Pleistocene refugia, Serranias del Burro.

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In a related study, Adams (2011) reported putative *Juniperus scopulorum* Sarg. from Colonia Pacheco, Mexico was very divergent from typical *J. scopulorum* in the Rocky Mountains. Analysis of nrDNA SNPs revealed that the Colonia Pacheco juniper was more similar to *J. blancoi* than *J. scopulorum*. However, the leaf essential oil was a little more similar to *J. scopulorum* than to *J. blancoi* (Adams, 2011). Adams (2011) concluded that the Colonia Pacheco juniper was the northern-most population of *J. blancoi*, but not typical due to hybridization with *J. scopulorum* in the Pleistocene.

A similar Mexico population of putative *J. scopulorum* exists in the Serranias del Burro Mountains, Coahuila. The author (with David Riskind) collected samples of putative *J. scopulorum* / *J. virginiana* at 1550m in 1977. Analysis of the leaf essential oils (Adams, 1983) indicated (Fig. 1) the S. del Burro population was most

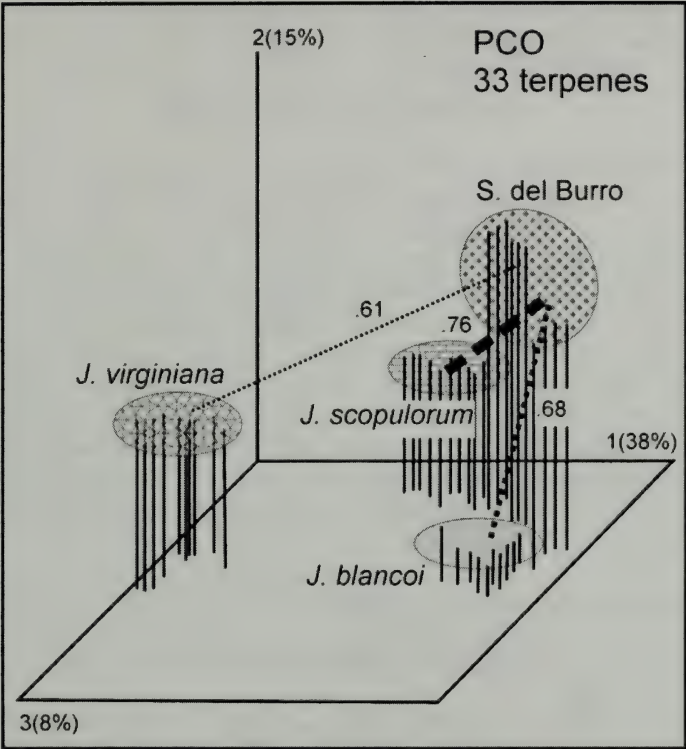


Figure 1. PCO based on leaf oil terpenes of *J. blancoi*, *J. scopulorum*, *J. virginiana*, and plants from Serranias del Burro (Coah., MX). The numbers next to the dashed lines are the similarities to the centroid of the clusters. Adapted from Adams (1983).

similar to *J. scopulorum* (Fig. 1). Adams (2008) treated the Serranias del Burro population as *J. scopulorum*. However, trees with two year old seed cones have not been found in the Serranias del Burro population, making identification as *J. scopulorum* problematical.

*Juniperus scopulorum* and *J. virginiana* are easy to differentiate based on SNPs from nrDNA (Adams, 2011). However, obtaining new specimens from the Serranias del Burro has been unsafe

due to the drug cartels operating in the area. The purpose of this paper is to report on the analyses of nrDNA from herbarium specimens from the Serranias del Burro.

## MATERIALS AND METHODS

Specimens used in this study were: *J. blancoi*: Adams 6849-6851 & 6903-6904, 2580 m, 7 km s of Carmona (s of El Oro) Mexico, Mexico; *J. blancoi* var. *huehuentensis* Adams 10247-10251, 3227 m, Cerro Huehuento, Durango, Mexico; *J. blancoi* var. *mucronata*, Adams 8453-8463, 1180 m, 19 km w of Maicoba, Chihuahua/ Sonora border, Mexico; *J. scopulorum*, Adams 2010-2024, 2012 m, Durango, CO, USA; *J. virginiana*, Adams 6753-6755, Hewitt, TX, USA; *J. scopulorum/ blancoi*, Adams 2512, Colonia Pacheco, Mexico; *J. scopulorum/ virginiana*: Adams 2433, 20° 01' 30"N; 102° 07' 30"W. ca. 1550 m, Serranias del Burro, Mexico, 20 Feb 1977, Adams 12493, Serranias del Burro, Mexico (ex TEX specimen 00124832, D. Riskind and T. F. Paterson #1933, 10 Apr 1976), Voucher specimens are deposited at BAYLU, Baylor University.

DNA isolation and analyses - See Adams (2011).

## RESULTS AND DISCUSSION

Sequencing nrDNA (1270bp) resulted in 13 SNPs (including indels) among the taxa. The samples from S. del Burro were separated from *J. virginiana* by 3 SNPs and from Colonia Pacheco (and n. Sonora) by 4 SNPs (Fig. 2). The S. del Burro plants are 8 SNPs removed from *J. scopulorum*, and 5 SNPs from *J. blancoi*. Recall that the leaf essential oils from S. del Burro were most like *J. scopulorum* then to *J. blancoi* (Fig. 1).

Plotting the minimum spanning network onto distribution maps (Fig. 3) gives a geographic perspective. Notice the proximity of S. del Burro to *J. virginiana*. The linkage of S. del Burro to *J. virginiana* (3 SNPs) is nearly the same as to Colonia Pacheco (4 SNPs).





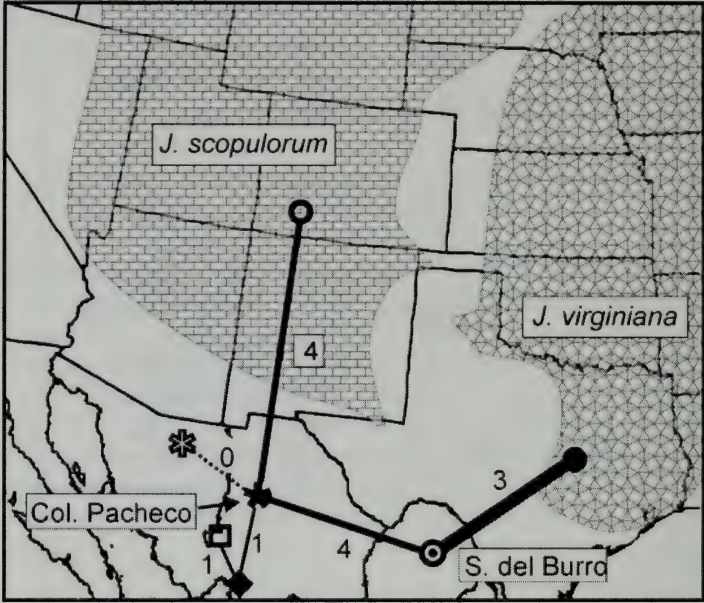


Figure 3. Minimum spanning network. Numbers next to the lines are SNPs. Symbols are defined in figure 2.

## CONCLUSIONS

It seems likely that during the Pleistocene ice ages, both *J. scopulorum* and *J. virginiana* repeatedly invaded northern Mexico as vegetation zones descended during cooler, more mesic periods. It is not surprising that Colonia Pacheco, northern Sonora and Serranias del Burro populations contain mixtures of genes of these species.

## ACKNOWLEDGEMENTS

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in 1977. A special thanks to Socorro Gonzales Elizondo for sharing both field data and specimens of *J. b.* var. *huehuentensis* from Cerro Mohinora and other data.

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**BIOLOGICAL STATUS OF *HEDEOMA DRUMMONDII*, *H. REVERCHONII* (LAMIACEAE) AND CLOSELY RELATED TAXA**

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**ABSTRACT**

Biological status of the closely related taxa, *Hedeoma reverchonii* and *H. drummondii* is reviewed with the conclusion that they are best treated as species, the latter possessing two intergrading regional categories: var. *drummondii* and var. *crenulata*. Distribution maps for the several taxa are provided. *Phytologia* 93(2): 174-180 (August 1, 2011)

**KEY WORDS:** *Hedeoma drummondii*, *Hedeoma reverchonii*, USA, Texas, Mexico

In the seminal paper of Epling and Stewart (1939), *Hedeoma drummondii*, a widespread highly variable, mostly narrow-leaved perennial of the U.S.A. and northern Mexico, and the more localized, mostly broad-leaved perennial of central Texas, *H. reverchonii*, were accepted as distinct species, separated from each other by a number of characters, most notably calyx and corolla size. The two workers did not recognize infraspecific categories in the taxa, but Irving (1980), who monographed the complex, recognized a var. *serpyllifolia* of the latter, this taxon having been recognized by earlier and subsequent workers. The following key sums up the major floral distinctions between the two species as recognized by Epling and Stewart:

- 1. Corolla tubes 9-14 mm long; calyx tubes 7-9 mm long.....**H. reverchonii**
- 1. Corolla tubes 4-8 mm long; calyx tubes mostly 4-7 mm long.....**H. drummondii**



The two workers provided a well-reasoned account of the acceptance of the taxa concerned, including synonyms, this summarized in the listings that follow.

**HEDEOMA DRUMMONDII** Benth., Labiat. Gen. Spec. 368. 1834.  
Typified by specimens collected by Berlandier near Monterrey, Nuevo Leon, Mexico.

*Hedeoma ciliata* Nutt. 1848

*Hedeoma sancta* Small 1899

*Hedeoma serpyllifolia* Small 1899

*Hedeoma longiflora* Rydb. 1909 (not *H. longiflora* Briq. 1897)

*Hedeoma camporum* Rydb. 1917

*Hedeoma ovata* A. Nelson 1904

Epling and Stewart note that *H. drummondii* throughout most of its range "is remarkably constant in the size and configuration of the flower parts, as well as the general aspect of the plant." Nevertheless, they believed "it is a practical impossibility to segregate these two species even approximately where they come together." They note further that numerous intermediates between the two taxa are found in central Texas, this presumably suggesting hybridization, but not stated as such. Indeed, they opined that such intermediates had served as the basis for Small's proposed species, *H. serpyllifolia*, *H. sancta* and *H. lata*.

Irving et al. (1979) more or less agreed with the assessments of Epling and Stewart regarding natural hybridization between *H. drummondii* and *H. reverchonii*, but the former authors believed that *H. serpyllifolia* could be recognized as a variety of the latter, as noted below.

I have reevaluated the status of *H. serpyllifolia* and conclude that the taxon is of hybrid origin, consisting of F1 individuals and/or back crosses of variable origin. Indeed, I have annotated 16 herbarium sheets on file at TEX that appear to be of hybrid origin, these obtained from 9 or more counties in central Texas. Most other plants in central Texas approach one or the other taxon and are perhaps best identified by the following key:

- Calyces 4-7 mm long; hairs on the tubes relatively short, mostly ca 1.2 mm long or less.....**H. drummondii**  
 Calyces 8-14 mm long; hairs on the tubes mostly ca 1.3 mm long or more.....**H. reverchonii**

**HEDEOMA REVERCHONII** (A. Gray) A. Gray, Syn. Fl. N Amer. (ed 2) 2: 460. 1886. Typified by a specimen collected in Brown Co., Texas by Reverchon in 1877.

*Hedeoma drummondii* var. *reverchonii* A. Gray 1878

Epling and Stewart noted this taxon to be "A variable perennial amply distinct in its extreme forms but merging almost indefinitely with *Hedeoma drummondii*."

Irving (1980), under my direction, undertook a doctoral study of *Hedeoma*. In this he largely followed the work of Epling and Stewart, like them noting that *H. reverchonii* and *H. drummondii* occur together in central Texas where they reputedly commonly form hybrids. Indeed, he bestowed the name *H. reverchonii* var. *serpyllifolia* (Small) Irving (typified by material from Kerr Co. Texas) upon somewhat intermediate plants, which Epling and Stewart included within *H. drummondii*, as noted in the above. Irving could as readily, in my opinion, have treated the taxon as a variety of the latter but preferred the former, for reasons not enumerated.

In my Atlas of the Vascular Texas Plants (Turner et al. 2003), I followed Irving's treatment, but treated his *H. reverchonii* var. *serpyllifolia* at the specific rank, largely on chemical grounds (Turner 1969), the taxon concerned bearing camphor-scented volatiles, its presumed compatriot, var. *reverchonii*, having lemon-scented volatiles. In hindsight, I think I erred in this disposition, for not enough was known at the time about the correlation of morphological characters with such scents (cf. Irving and Adams 1973). More recently I have studied the taxa anew, using herbarium sheets and limited field observations, and have come to the conclusion that the purely morphological treatment of the complex as rendered by Epling and Stewart is reasonably sound, and perhaps superior to that of Irving's study. Detailed DNA studies of the complex might prove me wrong.

Not noted in the above account is my recognition of *Hedeoma drummondii* var. *crenulata* Irving, this proposed by Irving in 1970, and typified by material from the state of San Luis Potosi, Mexico. Interestingly, Irving (1980) subsequently placed the taxon in synonymy within his broad concept of *H. drummondii*, but I accept the taxon as biogeographically distinct, this intergrading with typical var. *drummondii* in regions of contact (Fig. 2).

The following key should help identify the infraspecific taxa of *H. drummondii* recognized herein:

- Leaves mostly elongate-ovate, the margins to some extent crenulate;  
south-central Mexico.....var. **crenulata**  
Leaves mostly otherwise, the margins entire or nearly so; U.S.A. and  
north-central Mexico.....var. **drummondii**

In the above account I have treated *Hedeoma* as feminine, as opposed to masculine as treated by Irving and yet others, this resulting in the ending **a**, as opposed to **um**, to most of the descriptive taxa, this nicety called to my attention by Emer. Prof. Robert Harms with the following paragraph:

**Specific epithets of *Hedeoma*, from the Greek ἡδύς ‘pleasant to the taste or smell’ and the feminine noun ὀσμή ‘scent’ require the ending –a (*H. serpyllifolia*). These are frequently misinterpreted as Latin neuter nouns in the botanical literature, and accordingly assigned an–um ending ((*H. serpyllifolium*; cf. e.g., “List of Taxa in the Virtual Herbarium of the New York Botanical Garden”). This confusion of Greek feminines in μή (spelled with eta) and Greek neuters in μα (with alpha, e.g. *Nama*, from νᾶμα), both transliterated ‘ma,’ has a long history in the botanical nomenclature. (cf, Nicolson, D. H. 1994. Gender of Generic Names, Particularly Those Ending in -ma, in the ‘Names in Current Use’ List. Taxon 43:97-107.**

## ACKNOWLEDGEMENTS

My colleague, A. M. Powell of SRSC kindly reviewed the paper, providing helpful suggestions. Prof. Robert Harms, ex Linguistics Professor at the University of Texas, provided information regarding the feminine status of *Hedeoma*. Distribution maps are based upon specimens on file at LL-TEX and those cited by Irving in his doctoral study.

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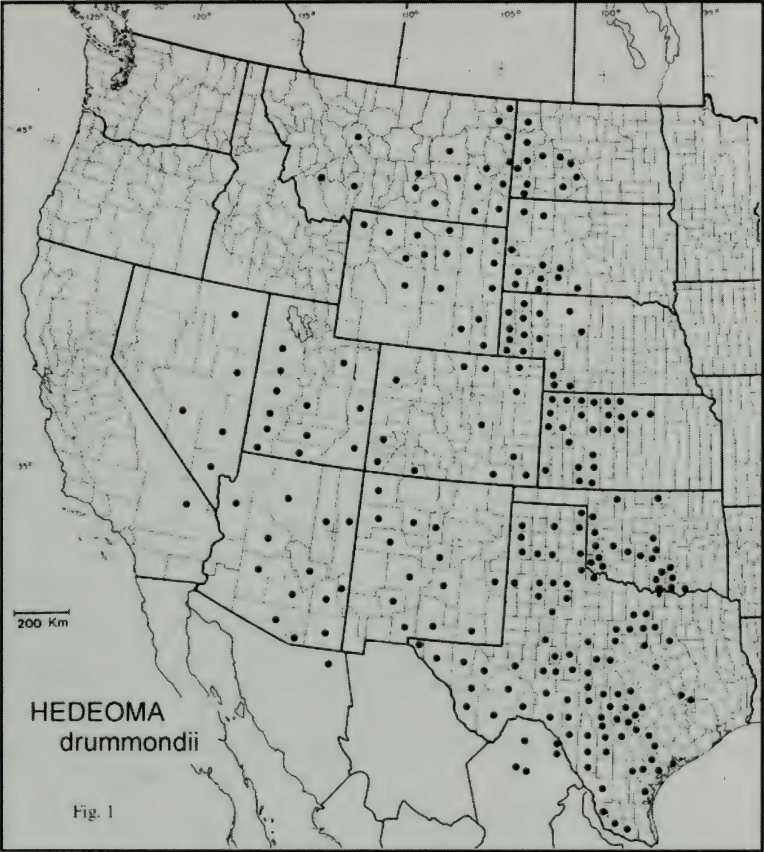


Fig. 1. Distribution of *H. drummondii* in the USA and closely adjacent Mexico.

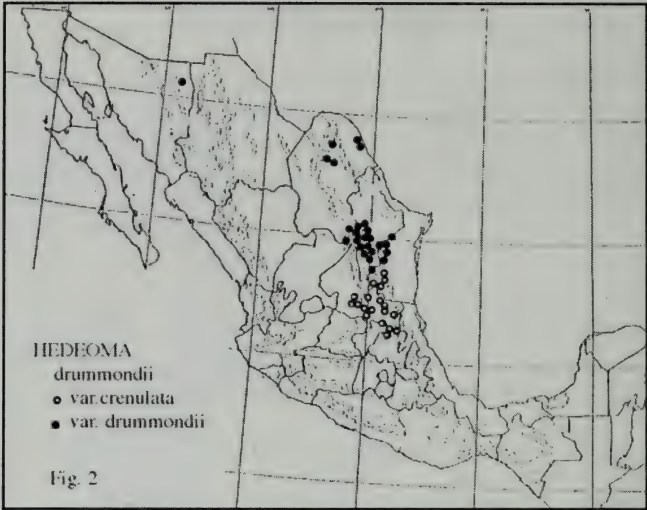


Fig. 2. Distribution of *H. drummondii* in Mexico.

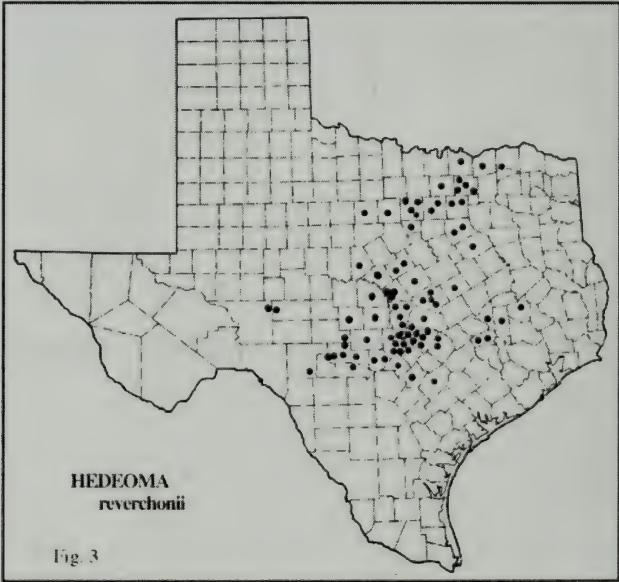


Fig. 3. Distribution of *Hedeoma reverchonii*.

SYSTEMATIC STUDY OF THE *DALEA NANA* COMPLEX  
(FABACEAE)

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ABSTRACT

The late Rupert Barneby (1977) rendered an excellent systematic treatment of *Dalea nana* Torr. in which the complex was treated as being but a single species having two, essentially sympatric, infraspecific taxa: var. *nana* and var. *carnescens* (Rydb.) K. & P. However, he suggested that the two populational systems might ultimately be treated as distinct species. The present populational study confirms the prescience of Rupert's observation and the var. *carnescens* is herein treated at the specific level as ***Dalea rubescens* Wats.**, a name first proposed for the taxon in 1882, this typified from material collected by Charles Wright in Trans-Pecos, Texas. Distribution maps of both taxa are presented, along with a discussion of the characters that separate them. *Phytologia* 93(2)181- 184 (August 1, 2011)

**KEY WORDS:** *Dalea*, *D. nana*, *D. n. var. carnescens*, *D. n. var. nana*, Texas, Mexico

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Key to species [largely adapted from Barneby (1977)]

- 1. Spikes relatively loose, at least in age; bracts mostly broadly ovate to elliptic-acuminate, mostly 1-2 times as long as wide; substrates mostly non-calcareous or sandy (rarely not).....**D. nana**
- 1. Spikes densely congested and cone-like; bracts lanceolate to ovate-lanceolate, mostly 3-5 times as long as wide; substrates calcareous, non-arenaceous (rarely not).....**D. rubescens**

**DALEA NANA** Torr. ex A. Gray, Mem. Amer. Acad. 11, 4 (Pl. Fendl. 1): 31. 1849.

**TYPE: OKLAHOMA. CIMARRON CO.:** "Willow Bar on the Cimarron." 28 Aug 1847, *Fendler 130* (GH) [data from Shaw, 1982].  
*Parosela nana* (Torr.) A. Heller

Barneby (1977) presented an exceptional analysis of the *D. nana* complex nearly all of which I agree with, except as to nomenclatural bestowals. He aptly noted that:

Racial differentiation in var. *nana* is apparently still active, expressed in small size-differences in the spikes and in length of the bracts, calyx-teeth, and androecia. Material from the upper Arkansas (nomenclaturally typical) and thence southwest to Rio Grande and on, sporadically, into extreme southeast Arizona, has relatively thick spikes (10-13 mm diam), long bracts (3.5-5.5 mm) and calyx-teeth (2.6-4.2 mm), and long androecia (mostly 8-10 mm). The populations on the Coastal Plain in southern Texas and adjoining Mexico, extending into central Texas, have narrower spikes (mostly 7-9 mm diam), correspondingly short bracts (2.5-4, rarely 4.5 mm), calyx teeth (2.2-3.4, rarely 3.8 mm), and androecia (6-8 mm). These differences represent, however, trends rather than accomplished modifications, coinciding for the most part with different life-zones, but are not yet fully established.

My study of the herbarium specimens at LL-TEX, SRSC largely confirm the observations of Barneby, and one might make a case for the recognition of an infraspecific category for the more southern populations of *D. nana*, with emphasis upon the characters called to the fore by Barneby, nearly all of these intergrading to some extent with the more typical, northwestern populations of the taxon. At least I could see little point in constructing varietal names for the populations concerned, but it will be interesting to see what DNA data might suggest.



**DALEA RUBESCENS** Wats., Proc. Amer. Acad. Arts 17: 369. 1882.  
LECTOTYPE: TEXAS. JEFF DAVIS CO.: Limpia Pass, NE of Fort Davis, Aug 1849, *Charles Wright 124* (GH).

*Parosela carnescens* Rydb.

*Parosela elatior* Vail, nom. illeg.

*Parosela lesueurii* Tharp & Barkley

*Dalea nana* var *elatior* A. Gray ex B.L. Turner, nom. illeg. (cf. Barneby 1977)

*Dalea nana* var. *carnescens* (Rydb.) K. & P.

*Dalea whitehouseae* Tharp & Barkley

Barneby (1977) notes that “Where it invades the territory of var. *nana*, as in the lower Rio Grande valley and in contiguous south corners of Arizona and New Mexico, the two forms remain, whenever I have seen them, segregated by soil preference, var. *carnescens* always on limestone rubble, caliche, or gypsum, and var. *nana* on acidic or neutral sands.”

After a lengthy discussion of the variability of the varieties *nana* and *carnescens*, Barneby (1977) concluded, “It is possible that they should be recognized as distinct species, but (as mentioned above) occasional morphological intermediates occur, difficult to assign to variety (but none, as yet, accompanied by habitat data).”

After considerable populational studies in central and Trans-Pecos, Texas I conclude that the two taxa concerned should be recognized at the specific level. Populations are readily identified, and when the two taxa are in close proximity they appear not to intergrade as a result of current hybridization, although it is possible that such might be found with more inclusive investigation. It is likely that ancient hybridization between the two taxa has occurred, this accounting for those plants that I assumed were possible extant hybrids of *D. nana* with *D. aurea*, (Turner 1959), this called to the fore by Barneby (1977).

According to Barneby, the species is named for the tendency of the yellow petals to turn fleshy pink or reddish upon drying. It is most readily recognized by its bracts, which are lanceolate and mostly 2.5-5.0 times as long as wide (vs broadly ovate and mostly 2.0-2.5

times as long as wide.), these nicely illustrated in Shinnery and Mahler's Illustrated Flora of North Central Texas.

### ACKNOWLEDGEMENTS

Distribution maps are based upon specimens on file at BRIT, SRSC and LL-TEX, for which I am grateful, supplemented by appropriate distributional data on the web pages (mainly USDA). Guy Nesom kindly reviewed the paper and offered helpful suggestions.

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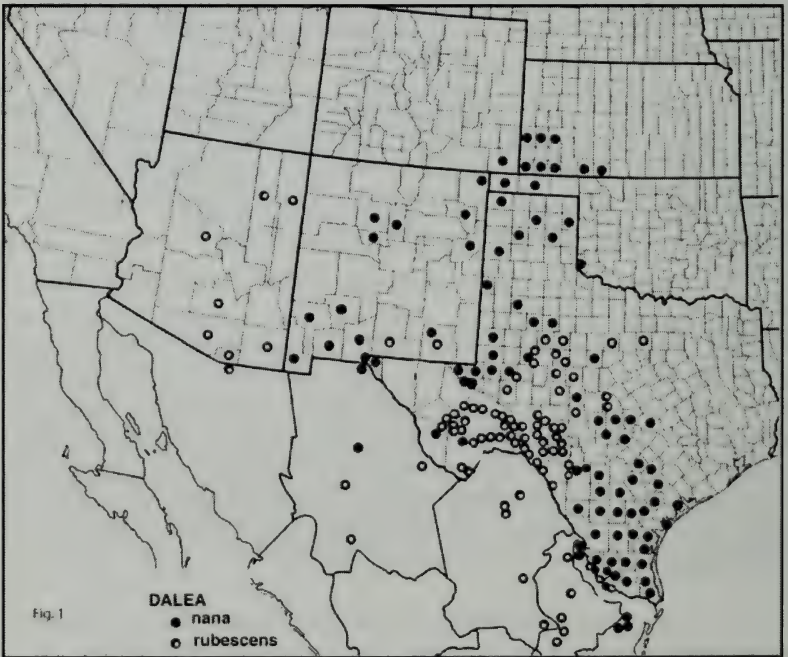


Fig. 1. Distribution of *Dalea nana*.

## TAXONOMY AND EVOLUTION OF *JUNIPERUS COMMUNIS*: INSIGHT FROM DNA SEQUENCING AND SNPs

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### ABSTRACT

Plants of *Juniperus communis* var. *communis* and var. *charlottensis*, var. *depressa*, var. *hemispherica*, var. *jackii*, var. *saxatilis* as well as *J. rigida* and *J. grandis* (outgroup) were sampled and SNPs from nrDNA, petN-psbM, trnD-trnT and trnS-trnG were examined. Several varieties were found to be very distinct: v. *jackii*, v. *hemispherica*, v. *oblonga*, and v. *charlottensis* with v. *jackii* as distinct from *J. communis* as from *J. rigida*. Sequence data support the colonization of *J. communis* from ancestral populations of the species in Asia, most likely using the Bering Land Bridge as opposed to long distance dispersal. Our results suggest that, potentially, two independent colonizations of the New World plus a secondary reverse movement from the New World to Kamchatka has taken place. *Phytologia* 93(2): 185-197 (August 1, 2011).

**KEY WORDS:** *Juniperus communis*, var. *charlottensis*, var. *depressa*, var. *hemispherica*, var. *jackii*, var. *megistocarpa* and var. *saxatilis*, Cupressaceae, geographic variation, nrDNA, petN, trnDT, trnSG, SNPs, Pleistocene migrations.

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*Juniperus communis* is the only species of *Juniperus* that occurs in both the eastern and western hemispheres (Adams, 2011). Analysis of Arctic populations of *J. communis* (Adams et al., 2003) revealed that these populations clustered by continent, with the populations in Greenland and Iceland showing the highest affinities to populations from Europe and not to those from North America (Fig. 1). Adams et al. (2003) concluded that the post-Pleistocene populations on Greenland

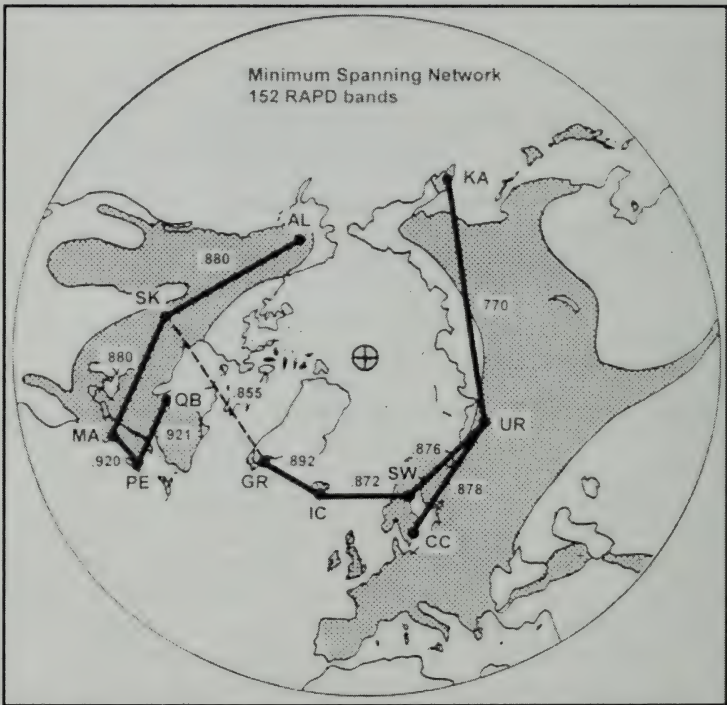


Figure 1. Minimum spanning network showing that the North American *J. communis* var. *depressa* and var. *megistocarpa* populations link together and all the *J. communis* populations from the e. hemisphere link together (Adams et al., 2003). The dashed line is the minimum link between eastern and western hemisphere populations. Shaded area shows the distribution of *J. communis*.



and Iceland came from Europe and not North America. Adams and Pandey (2003) analyzed *J. communis* and its varieties by use of RAPDs and found considerable variation, but several of the varieties were not discernable.

Adams and Nguyen (2007) collected additional samples of putative *J. c. var. saxatilis* from the Pacific northwest, *J. c. var. jackii* from NW California and *J. c. var. depressa* from the southernmost locations in North America (Mt. Charleston, Nevada and Mt. Satula, North Carolina). They found the major trend among the taxa was the separation of the eastern hemisphere plants (*J. communis* var. *communis*, *J. c. var. saxatilis*, and putative *J. c. var. saxatilis*, Kamchatka) from the western hemisphere plants (*J. c. var. depressa*, *J. c. var. jackii*, *J. c. var. megistocarpa*, and putative var. *saxatilis*). The resolution of *J. c. var. jackii* (and plants from Mt. Hood) was in contrast to the report by Ashworth, et al. (1999, 2001).

More recently, Adams (2008) examined nrDNA SNPs in varieties of *J. communis* in North America and found *J. c. var. jackii* to be very distinct (Fig. 2) along with the juniper from Queen Charlotte Island (recognized as *J. c. var. charlottensis* R. P. Adams). Interestingly, *J. c. var. depressa* and *J. c. var. saxatilis* (Japan) were found to be identical in their nrDNA.

The purpose of this paper is to report on the taxonomy and evolution of *J. communis* based on a more comprehensive taxon and data sampling using all varieties of *J. communis* and nrDNA, petN-psbM, trnD-trnT and trnS-trnG sequence data.

## MATERIALS AND METHODS

Specimens used in the present study: *J. communis* var. *communis*: Adams 7846-7847, Sweden; *J. c. var. charlottensis*: Adams 10304-10308, Queen Charlotte Island, BC, Canada; *J. c. var. depressa*: Adams 7802-7804, Victor, CO, USA; *J. c. var. hemispherica*: Adams 9045-9046, Mt. Etna, Sicily; Adams 7589-7590, Sierra Nevada, Spain; *J. c. var. jackii*: Adams 10287-10291, serpentine, Del Norte Co., CA; USA; *J. c. var. megistocarpa*: Adams 8575-8576, Magdalen Island, Quebec, Canada; *J. c. var. nipponica*: Adams 8579, 8690, Japan (ex Jin

Murata); *J. c.* var. *oblonga*: Adams 8764-8765, Lake Sevan, Armenia; *J. c.* var. *saxatilis*: Altair Mtns., Mongolia; Adams 7194-7195, Adams

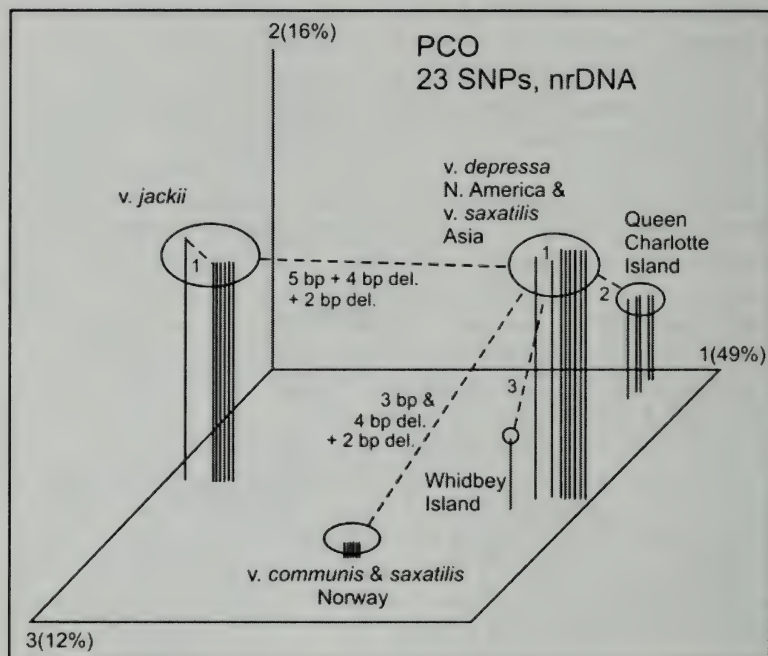


Figure 2. PCO of 58 individuals based on 23 SNPs. Identical bars, closely spaced indicate no variation among these individuals. The bars in the largest group are symbolic, as that group contains 27 individuals!

11206, 11207 Norway; Adams 10890-10893, Redfish Lake, Idaho; Adams 9181-9183 Esso, Kamchatka, Peninsula, Russia (ex. J. W. Leverenz); Adams 8686-8687, Hokkaido, Japan (ex Naotoshi Yoshida); Adams 11088, 11090, Sakhalin Island, Russia (ex Hideki Takahashi 31164, 31094); Adams 10185-10186, Urup Island, Kurils, Japan (ex M Ohara sn, Hideki Takahashi 22211); *J. rigida*: Adams 8544-8545, (ex Jin Murata), Honshu Island, Japan; *J. grandis*: Adams 11963-11964, Meyers, CA. Voucher specimens are deposited at the Baylor University herbarium (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at  $-20^{\circ}\text{C}$  until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

**PCR amplification:** Amplifications were performed in 30  $\mu\text{l}$  reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15  $\mu\text{l}$  2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200  $\mu\text{M}$  each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM  $\text{MgCl}_2$  according to the buffer used) 1.8  $\mu\text{M}$  each primer. See Adams and Schwarzbach (2011) primers utilized. The primers for nrDNA, petN-psbM, trnD-trnT, and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). See Adams and Schwarzbach (2011) for band purification and sequencing procedures as well as data analysis.

## RESULTS AND DISCUSSION

Concatenation of nrDNA, petN-psbM, trnD-trnT and trnS-trnG sequences resulted in 3603 bp of data. A NJ tree (Fig. 3) shows several surprising results. *Juniperus communis* var. *jackii* (nw USA) grouped outside *J. communis* and its varieties, with *J. rigida* being more closely related to the other *J. communis* varieties (Fig. 3). *Juniperus c.* var. *jackii* has a very unusual habitat (serpentine and volcanic lava), morphology and leaf oils. Adams et al. (2010) reported that var. *jackii* leaf essential oil is low in  $\alpha$ -pinene, high in  $\delta$ -3-carene, and terpinolene (as found in *J. c.* var. *saxatilis* from Switzerland). In addition, var. *jackii* has 6 diterpenoids that are not found in any other *J. communis* variety, except in *J. c.* var. *saxatilis* from Switzerland (Adams et al. 2010). It is not surprising that it is distinct from other North American members of *J. communis*, but to be as distinct as *J. rigida* is surprising.

In contrast, *Juniperus communis* var. *megistocarpa* is not resolved as a distinct clade. Yet, it has the most distinctively large female cones in the species and an unusual habitat, occurring on sand dunes on islands and seashores and a somewhat unusual leaf essential oil composition (low in  $\delta$ -3-carene and  $\beta$ -phellandrene, very high in limonene, no  $\alpha$ -terpinyl formate or germacrene B, and the presence of

(Z,E)-farnesal, Adams et al. 2010). In addition, several other varieties could not be resolved: *J. c.* var. *communis* (Sweden). *J. c.* var. *saxatilis* (Norway, Idaho) and *J. c.* var. *depressa* (Colorado, USA). *Juniperus c.* var. *hemispherica* (Mt. Etna, Sicily), previously not considered distinct in its morphology or RAPDs (Adams, 2008), has good support as a distinct clade, along with putative *v. saxatilis* shrubs from the Sierra

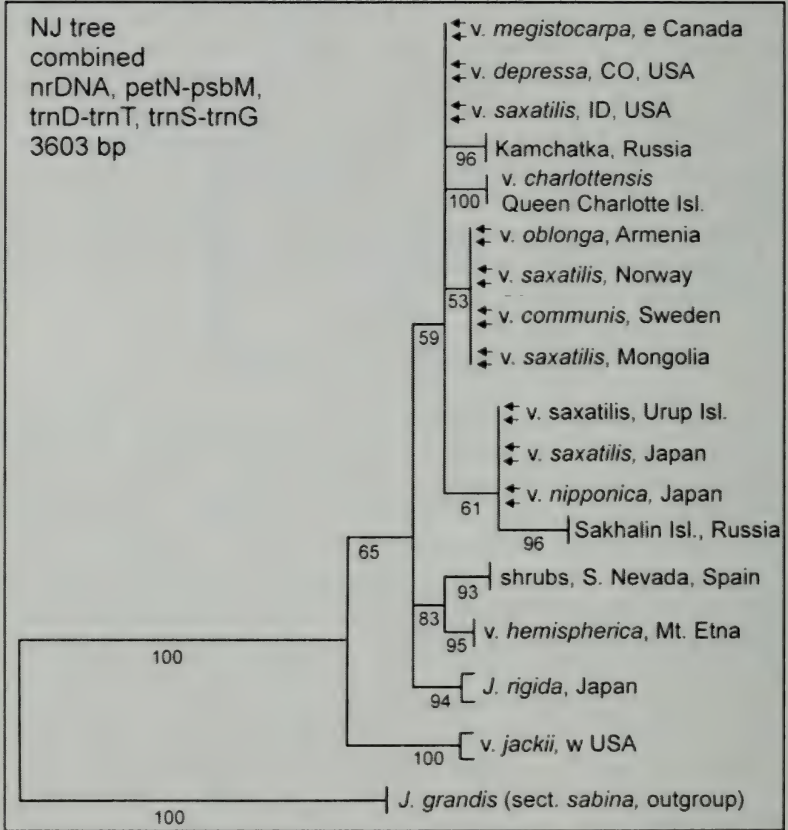


Figure 3. NJ tree based on 3603 bp of sequence data. Numbers at the branch points are bootstrap percentages (1000 reps.). Branches with less than 50% support are not shown. Various accessions with unknown affinities from Kamchatka, Russia, Sakhalin Island, Russia and from S. Nevada, Spain are labeled with their geographical identity.



Nevada, Spain (Fig. 3). The shrubs from the Kamchatka Peninsula, Russia are also in a well supported clade as are the putative var. *saxatilis* shrubs from Sakhalin Island (Fig. 3). Taxa referred to as var. *saxatilis* are found in three clades: with European-Asian *J. communis*; with North American *communis* and with Japanese taxa. *Juniperus c.* var. *saxatilis* was described from Russia and the name is commonly applied to shrubs having leaves with the stomatal band 2-3x as wide as the green side bands (Adams, 2011). Although putative var. *saxatilis* from North America and Japan satisfy those morphological characteristics, it appears (Fig. 3) that three taxa are present and var. *saxatilis* (*sensu stricto*) only occurs in Europe and central Asia.

The Bayesian Tree (Fig. 4) is very similar to the NJ tree (Fig. 3). Additional trees (parsimony and maximum likelihood) gave similar, but less resolved trees. As the *communis* varieties are likely freely hybridizing and are of very recent origin (Mao et al. 2010), tree building programs may not be appropriate for these kinds of data.

The NJ tree does not take into account indels and indels are very common in the cpDNA regions sequences. To assess the total mutations, indels were coded. Analysis of the 3603 bp data set revealed 135 mutational events (nucleotide differences plus indels). The nucleotide polymorphisms (ignoring single events among the accessions) plus indels are noted as SNPs (although technically SNPs refers to nucleotide differences). A minimum spanning network shows (Fig. 5) the major trend to be the tremendous differentiation of var. *jackii* (19 SNPs!) as well as a closely related species, *J. rigida*, by 20 SNPs.

As previously mentioned, var. *jackii* has a number of differences from other *communis* taxa. *Juniperus c.* var. *communis* (Sweden), the Norway shrubs (locally called *J. c.* var. *saxatilis*) and the Mongolian var. *saxatilis* shrubs are separated from other populations by 5 to 7 SNPs. The Mongolia shrubs are scarcely distinct from var. *communis* (2 SNPs, Fig. 5). The very long leafed plants from Armenia differed by 5 SNPs, lending support for the recognition of var. *oblonga*.

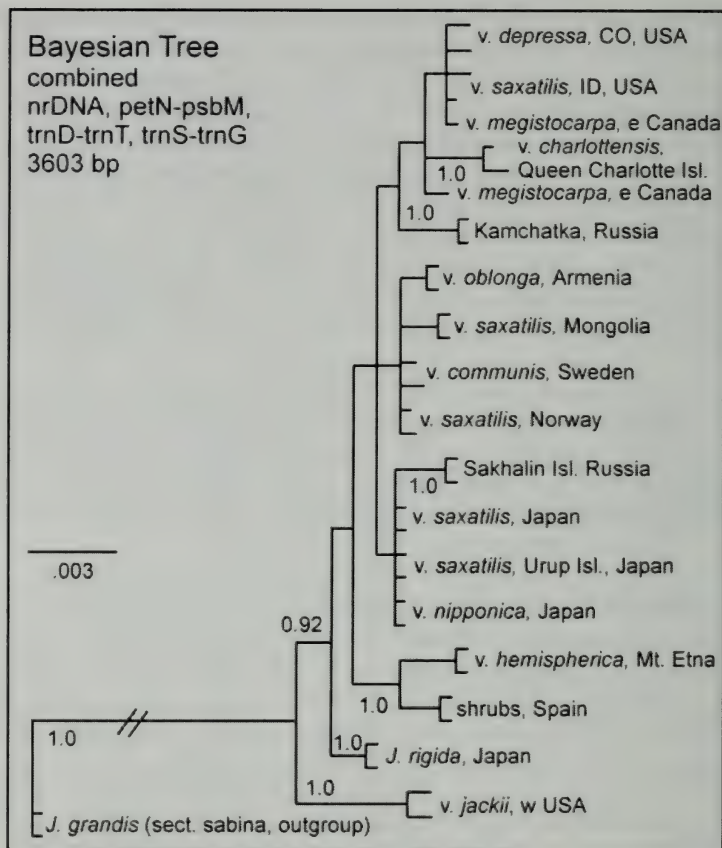


Figure 4. Bayesian tree based on 3603 bp of sequence data. Only those posterior probabilities greater than 0.90 are shown.

Adams and Pandey (2003) examined RAPDs from *J. c.* var. *hemispherica* from the type locality (Mt. Etna) and found them to be very similar to typical *J. c.* var. *communis* (Sweden). Based chiefly on these data, Adams (2011) treated var. *hemispherica* as a synonym of *J. c.* var. *communis*. The present sequence data gives support for the recognition of var. *hemispherica*, with its 7 SNPs differences from the shrubs in the Sierra Nevada, Spain and 11 SNPs differences from *J. c.*

var. *communis* (Fig. 5). Of considerable interest are the unusual shrubs (collected as var. *saxatilis*) from the Sierra Nevada, Spain. These differ by 9 SNPs from *J. c. var. communis* and 7 SNPs from var. *hemispherica* (Fig. 5).

The putative var. *saxatilis* (glaucous stomatal band 2-3x as wide as green side bands) from the western United States (Idaho) differs by 2 SNPs from *J. c. var. depressa/megistocarpa* (Fig. 5) and is not as closely related to typical var. *saxatilis* from the eastern hemisphere. In addition, the Kamchatka shrubs (called var. *saxatilis* by Adams 2008, 2011) appear to be more related to var. *depressa/megistocarpa* than to *J. c. var. saxatilis* from Europe and Asia (Fig. 5).

To examine the geographical variation in *J. communis*, a minimum spanning network was plotted onto the distribution map (Fig. 6.). In general, there appears to be considerably less variation in the western than in the eastern hemisphere. This may reflect the more recent colonization of the western hemisphere by *J. communis* (Mao et al. 2010).

A second trend is that the linkage of the western hemisphere to the eastern hemisphere by the Bering Land Bridge (BLB) seems stronger than across the Atlantic (Fig. 6). Mao et al. (2010) argued that *J. communis* could have come to the western hemisphere by land bridges or by long distance dispersal by birds (see Adams and Thornburg, 2010 for a review of seed dispersal in *Juniperus*). The present data give some support that *J. communis* came to the western hemisphere by the BLB.

The Kamchatka population is more closely related to North America plants than to nearby populations on the Urup and Sakhalin Islands (Fig. 6); This may reflect recent secondary migration from Alaska to Kamchatka during one of the several land bridges during the Pleistocene.

The divergence of var. *hemispherica* from Mt. Etna and var. *communis* from Sierra Nevada, Spain is rather large (Fig. 6). Additional research is being conducted to determine the extent of the divergence of Spanish *J. communis* in that region.

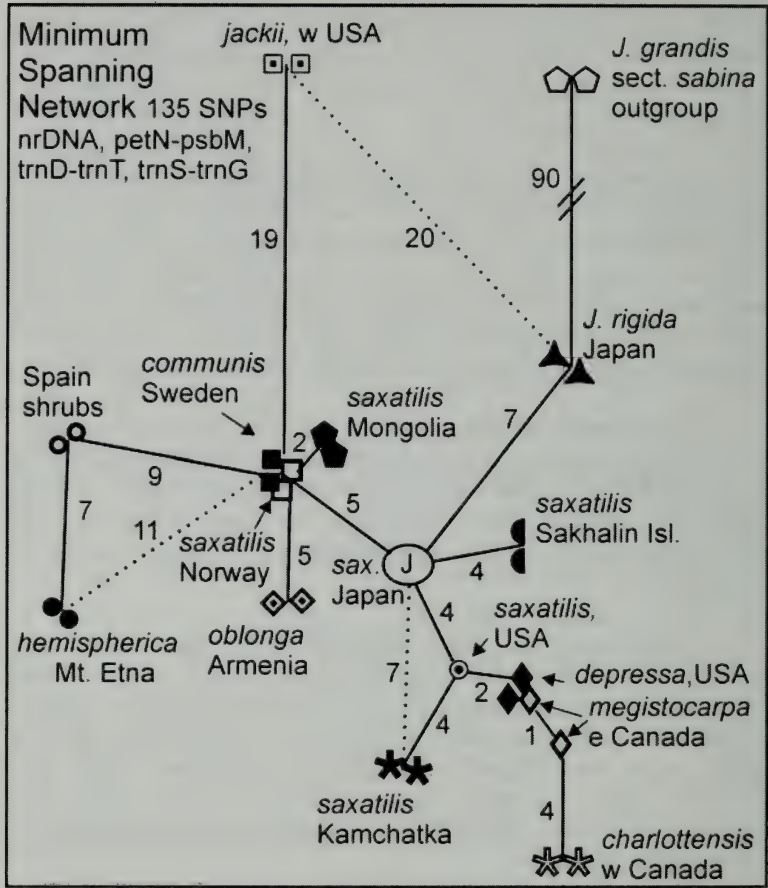


Figure 5. Minimum spanning network. The numbers next to the lines are the number of mutational events. The dotted lines are the second nearest link.

The distinct nature of *J. c.* var. *jackii* is shown in that its nearest links are 19 SNPs removed from *J. communis* (Sweden) and *J. c.* var. *charlottensis* (w Canada). Recall that var. *jackii* is 20 SNPs removed from *J. rigida* (Japan). Although var. *jackii* appears to be part



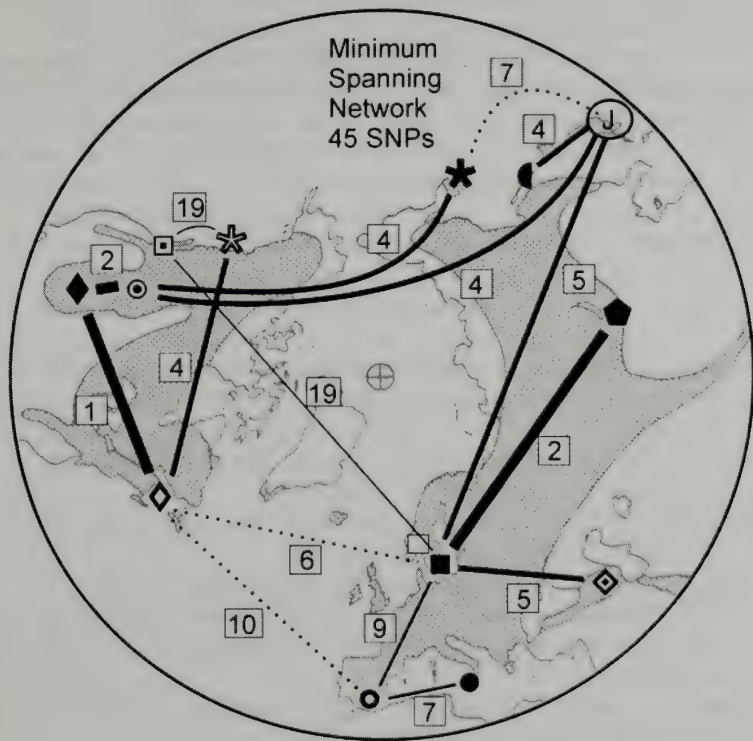


Figure 6. Minimum spanning network based on 45 SNPs. The width of the lines is proportional to the strength of the link. The numbers next to the links are the number of SNPs separating the nodes.

of the *communis* - *rigida* group, it is quite distinct in the sequences analyzed in this study. It seems possible that var. *jackii* (or its ancestor) may have been the first taxon of section *Juniperus* to migrate to North America. The *J. communis* var. *depressa* complex in North America is not very variable and appears closely related to *J. communis* from Japan. This complex appears to be a rather recent migration (Pleistocene?). Additional studies (in progress) are needed to elucidate the phyletic past of var. *jackii*.

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## LOW VARIABILITY OF DNA FINGERPRINTS OF TEXAS SNOWBELLS: CONSERVATION IMPLICATIONS

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### ABSTRACT

Texas snowbells (*Styrax platanifolius* var. *texanus*) is one of the most threatened native Texas plants. A preliminary study using DNA fingerprinting (RAPDs) was performed on plants from three natural populations. Almost no genetic variation was found, either within or between these three populations. Implications for conservation are discussed. *Phytologia* 93(2):198-202 (August 1, 2011)

**KEY WORDS:** *Styrax platanifolius* var. *texanus*, Texas snowbells, RAPDs, conservation.

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Texas snowbells (*Styrax platanifolius* Engelm. ex Torr. var. *texanus* (Cory) B. L. Turner) is a shrub or small tree that grows out of crevices on steep limestone bluffs, rock ledges, or cliff faces along creeks. It can also grow in the dry gravel of streambeds. The flowers are clustered at the end of the branches and hang upside down. The flowers dangle and look like small white bells, thus the common name, snowbells. Texas snowbells are endemic to an area of a 64 km radius within the southwestern Edwards Plateau of Texas. All but two populations are on private land. The natural populations are represented by 15 sites, varying from one mature plant to about 350 individuals (seedlings, juveniles, and reproductive plants). The sizes of these sites range from a few square meters to about 100 acres. Texas



snowbells are in cultivation at the San Antonio Botanical Center and in seed storage at the Wildflower Center in Austin (as part of their Center for Plant Conservation collection). Several dozen new populations representing hundreds of individuals have been reintroduced on private lands through the efforts of J. David Bamberger, his staff, and volunteers.

Fritsch (1996) examined 24 isozymes from 36 individuals of *S. platanifolius* in west Texas and found low levels of variation both within and among three populations. He concluded that there was no evidence of polyploidy and the gene flow between populations appeared to be high (but with a reservation that the methods may not be accurate). Later, Fritsch (1997) recognized three subspecies (*stellatus*, *texanus*, and *youngiae*), these subsequently treated as varieties by Turner and Nesom (2000). Fritsch (2001) further examined the phylogeny of *Styrax* using nrDNA and cpDNA data and found no differences between *S. platanifolius* var. *mollis*, var. *stellatus* and var. *texanus*.

The purpose of this paper is to report on a preliminary study of variation within and between three natural populations of Texas snowbells using RAPDs (Random Amplified Polymorphic DNAs).

## MATERIALS AND METHODS

Specimens collected: leaves were collected from 10 trees in three populations of var. *texanus* by J. Poole (10 Nov 2003: C1-C10 (= lab # *Adams 10091-10101*), Corbin property, sse of Dolan Falls, Val Verde Co., Texas; DF2, 31, 41, 45, 108a, 111, 123, 129, 141, 154, 164 (= lab # *Adams 10101-10111*), Dolan Falls Preserve, Val Verde Co., Texas; GV1, 6, 7, 7-3, 8, 17, 20, 25, 25a (= lab # *Adams 10112-10120*), Greenwood Valley, Ranch, Edwards Co., Texas.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA). The RAPD analyses follow that of Adams and Demeke (1993). Sixteen ten-mer primers were

purchased from the University of British Colombia (5'-3'): 116, 153, 184, 204, 212, 218 239, 244, 250, 265, 338, 347, 375, 389, 413, 431.

PCR stock solutions (Taq, primer, buffer) were made in bulk so that all the PCR reaction tubes for a primer were prepared using the same bulk stock. This is a critical factor for minimizing variation in band intensities from sample to sample (see Adams, Flournoy and Pandey, 1998, for protocols to minimize PCR band variation). PCR was performed in a volume of 15  $\mu$ l containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.0 mM  $MgCl_2$ , and 0.1% Triton X-100, 0.2 mM of each dNTPs, 0.36  $\mu$ M primers, 0.3 ng genomic DNA, 15 ng BSA and 0.6 unit of Taq DNA polymerase (Promega). A negative control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). Samples were run in duplicate to insure reproducibility (Adams, Flournoy and Pandey, 1998). A temperature profile was obtained for each well of the thermocycler to be sure that no variation existed among wells in the heating cooling block. The thermal cycle used was: 94°C (1.5 min) for initial strand separation, then 40 cycles of 40°C (2 min), 72°C (2 min), 91°C (1 min). Two additional steps were used: 40°C (2 min) and 72°C (5 min) for final extension. The temperature inside a PCR tube with 15 $\mu$ l buffer was monitored with a temperature probe for each step for each of the 40 cycles (Adams, Flournoy and Pandey, 1998) to insure that each cycle met temperature specifications and that each PCR run was exactly the same. Amplification products were analyzed by electrophoresis on 1.5% agarose gels, 75V, 55 min, and detected by staining with ethidium bromide. The gels were photographed over UV light using Polaroid film 667 and scanned to digital images. The digital images were size normalized in reference to pGem® DNA size markers before band scoring.

## RESULTS AND DISCUSSION

The 16 primers utilized resulted in 120 bands. In general, there was very little if any variation among individuals or between populations. Figure 1 shows bands for primers 218, 244, and 431. Notice that most of the individuals have identical DNA bands. RAPD 244 reveals 5 mutations scattered among the 3 populations. The other

13 primers resulted in similar patterns with little or no variation among individuals. Fritsch (1996) found very low diversity in isozymes in *S. platanifolius* populations in west Texas and concluded they had undergone a genetic bottleneck. The present preliminary data seems to confirm his data.

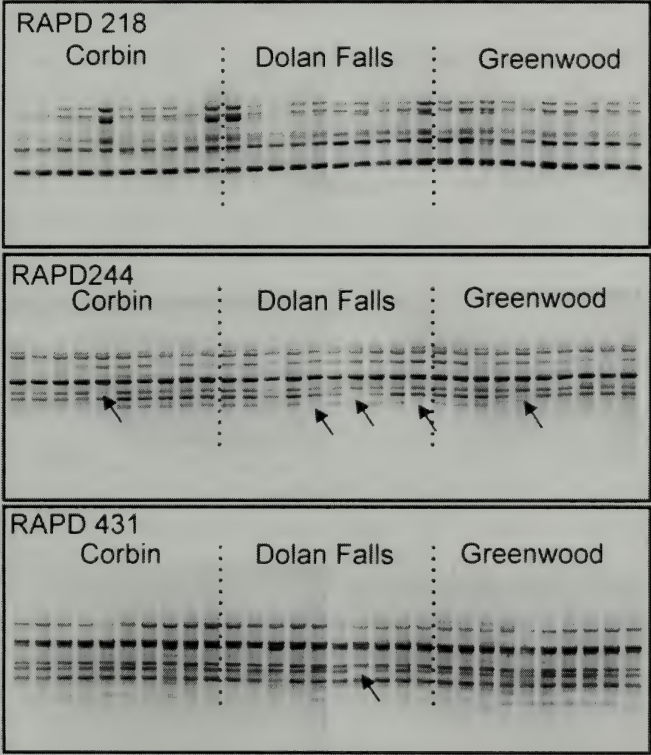


Figure 1. Banding patterns for individuals from three populations for three RAPD primers. The arrows indicate missing bands.

CONCLUSIONS

The present data, although preliminary, are concordant with the isozyme data (Fritsch, 1996) that there is very little genetic variation among Texas snowbells. It appears that conservation of several natural

populations will not conserve genetic variation. However, maintaining several natural populations guards against a catastrophic extinction of Texas snowbells and might lead to the accumulations of genetic mutations in the future to diversify the genetic base.

### ACKNOWLEDGEMENTS

This research was supported in part with funds from Baylor University. We are grateful to The Nature Conservancy of Texas and the private landowners for allowing us to collect the leaf samples, and to Texas Parks and Wildlife Department and the Wildflower Center for staff time to obtain and process the collections.

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***JALTOMATA ATQUIPA* (SOLANACEAE): A NEW SPECIES OF  
SOUTHERN PERU**

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**ABSTRACT**

A new species, *Jaltomata atiquipa* Mione & S. Leiva G. (Solanaceae), is described from Lomas de Atiquipa, Department Arequipa, Peru. Photographs of the novelty and a key to the *Jaltomata* Schltldl. species of the South American lomas formations are provided. *Phytologia* 93(2):203-207 (August 1, 2011)

**KEY WORDS:** *Jaltomata atiquipa*, lomas, lomas formation, Peru, Solanaceae

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*Jaltomata* is a diverse genus of about 60 species, growing from Arizona, USA south into Bolivia, on the Galápagos Islands (1 species) and in the Greater Antilles (1 species). Most of the species grow, and most of the morphological diversity can be found, in the Andes mountains of South America. Recent fieldwork has led to the discovery of numerous species (Leiva et al. 2007, 2008; Mione and

Spooner 2010; Mione et al. 2007). The purpose of this work is to describe a new species encountered during fieldwork in southern Peru, and to provide the first key to the *Jaltomata* species of the South American Lomas Formations. See Dillon (2005) for details about the Solanaceae of the Lomas Formations of South America.

*Jaltomata atiquipa* is distinguished from the species to which it is most similar, *J. diversa* (J. F. Macbr.) Mione, by the presence of a peduncle, pedicels and peduncles finely pubescent to glabrous; additionally the largest leaves are basally truncate. *Jaltomata diversa*, on the other hand, lacks a peduncle, the pedicels are densely pubescent, and leaves are rounded at the base. Furthermore, *J. atiquipa* is known only from a Lomas Formation, a low elevation fog-dependent community, while *J. diversa* is widespread in southern Peru at elevations between 2600-3600 m and does not grow in Lomas Formations.

***Jaltomata atiquipa* Mione & S. Leiva G., sp. nov. TYPE: Peru.**

Department Arequipa, Prov Caraveli, Lomas de Atiquipa, 15° 45' 38.3" S to 15° 45' 36.1" S X 74° 22' 24.5" W to 74° 22' 36" W, 842 – 996 m, among shrubs and in protection of a larger shrub, 17 Jan 2010, T. Mione, S. Leiva G. and L. Yacher 804 (holotype, F; isotype, HAO). Figures 1 – 4.

Frutex ad 1.5 m altus; folia grandiora basaliter truncata; inflorescentia 7 (-8)-floris, pedunculus ad 21 mm longus; corolla alba, crateriformis, pentaloba, ad 15 mm diametro ubi pressa et 10 maculas virides basi ferens; stamina 4 mm longa; styli albida, 6 mm longa.

Shrub to 1.5 m high, older branches/axes hollow, brown, glabrate with lenticels, to 1 cm diameter. Young stems green, puberulent, terete. Leaves (Fig. 3) alternate, sometimes geminate, the blade ovate, the larger leaves basally truncate (Fig. 3), sometimes basally oblique, the apex acute or subacute, sometimes acuminate, the margin subentire to toothed; younger leaves puberulent, becoming glabrate, the blade to 11 X 13 cm; petiole to 4.2 cm. Inflorescence axillary, to 7 (-12) flowered (Fig. 4). Peduncle (to 21 mm) and pedicel (to 11 mm) terete, green, finely pubescent to glabrous. Calyx green (Figs. 2, 4), planar at anthesis, 8 – 11 mm diam., the lobes triangular,

the margins ciliate. Corolla (Figs. 1, 2 & 4) white, crateriform, 5-lobed, 12 to 15 mm in diameter, 10 green maculae at base of corolla, the hairs of two types: 1) non-glandular uniseriate finger hairs to 0.8 mm long, and 2) stalked multicellular-headed glands 75 – 85 micrometers long (as illustrated in Mione and Serazo 1999). Stamen (Fig. 1) 4 mm long including anther, the filament whitish and glabrous except for hairs 0.2 mm long at base; anthers 1.1 - 1.4 mm long, cream-colored prior to dehiscence, emucronate. Pollen grains 25 – 30  $\mu\text{m}$  in diameter ( $n = 24$  grains, mean 28.67  $\mu\text{m}$ ), 58,250 – 99,500 per androecium ( $n = 5$  flowers, mean 69,950 grains). Gynoecium glabrous, except for stigma papillae 0.03 mm long. The style (Fig. 2) whitish, 6 mm long; the stigma green, capitate (Fig. 2) with a shallow medial groove, exerted beyond the dehiscent anthers. The ovary green; the disk (orangish in color) approximately 60% the height of the ovary. Ovules 81 – 94 per ovary ( $n = 4$  flowers, mean 89). Mature berries and seeds not seen, most likely orange and subspherical.

**Etymology.** The species epithet honors the people of the town of Atiquipa, near the type locality.

A key to the *Jaltomata* of the Peruvian lomas formations:

- 1. Floral nectar red/orange.....2
- 1. Floral nectar transparent.....4
- 2. Corolla tubular; shrub.....*J. umbellata* (R. & P.) Mione & M. Nee
- 2. Corolla very broadly campanulate; herbaceous or herbaceous and woody only at base.....3
- 3. Corolla lacking maculae.....*J. aspera* (R. & P.) Mione
- 3. Corolla having 10 green maculae.....Weigend & Forther 97/701, undescribed
- 4. Branches villous with gland-tipped hairs.....*J. hunzikeri* Mione
- 4. Branches essentially glabrous or villous on lower half.....5
- 5. Filaments essentially glabrous.....*J. atiquipa*
- 5. Filament villous along approximately the lower half.....6
- 6. Stamens included (inside of corolla); corolla 5-lobed.....  
.....*J. lomana* Mione & S. Leiva
- 6. Stamens exerted; corolla 10-lobed..*J. truxillana* S. Leiva & Mione

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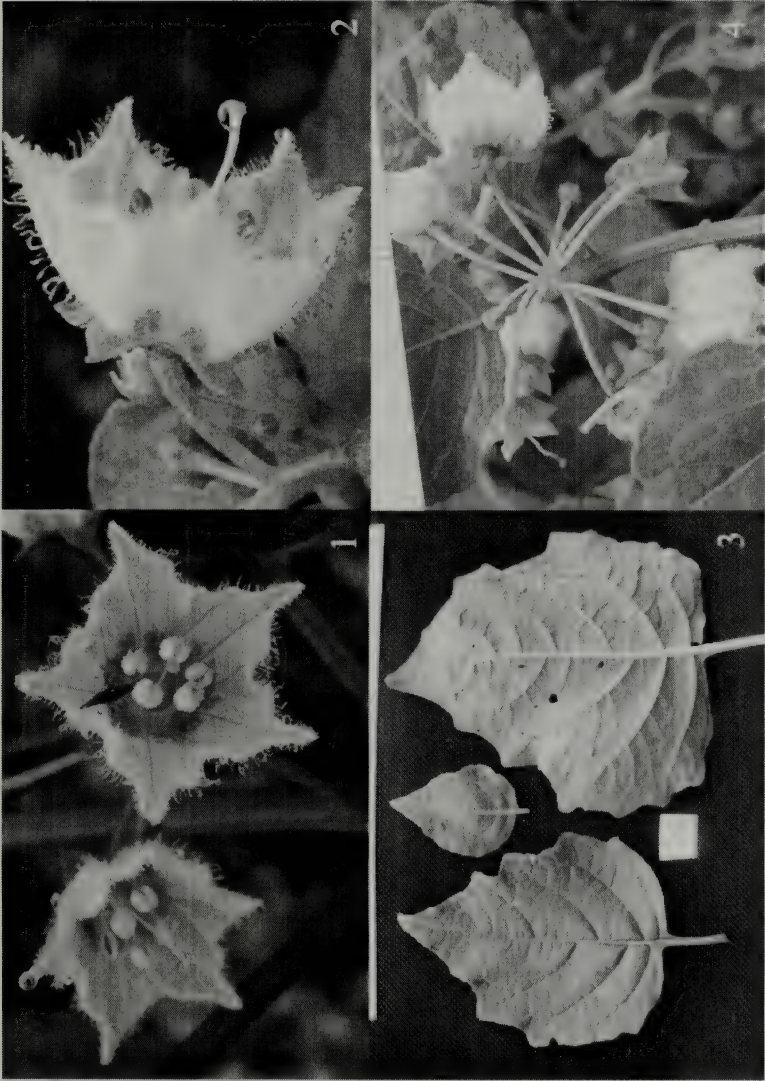
We thank Michael O. Dillon and David M. Spooner for valuable comments on the manuscript, the curators of BH, COLO, F, K, MO, NY and US for loan of specimens, Kanchi N. Gandhi for the Latin diagnosis, and Stephanie J. Sergi for preparation of the figures.

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Facing Page: Figure 1: Flowers of *Jaltomata atiquipa*, frontal view; bottom anther of right flower in the process of dehiscent; all other anthers dehiscent. Figure 2: Side view of flower with exerted stigma/style; two dehiscent anthers (brownish) showing; green maculae visible in throat of corolla in figure 1 also visible here at base of corolla. Figure 3: Leaves on left and in middle showing adaxial face; leaf on right showing abaxial face. Figure 4: Inflorescence; corolla of flower at left abscised leaving the persistent style. In figures 1 and 2, corolla 12 – 15 mm in diameter; units along top edge of figures 3 and 4 are mm. Photos by T. Mione.





**TAXONOMY OF INFRASPECIFIC TAXA OF *ABIES*  
*CONCOLOR*: LEAF ESSENTIAL OILS OF VAR. *CONCOLOR*  
AND VAR. *LOWIANA* - ERRATA**

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**ABSTRACT**

Previously (Adams et al, 2011), the volatile leaf oils of *Abies concolor* were reported. Unfortunately, due to a sample-numbering error, the composition of the New Mexico population was incorrect. This paper reprints the original paper with the corrected data. The leaf essential oils of *Abies concolor* var. *concolor* and var. *lowiana* had large amounts of  $\beta$ -pinene (34-52%). The oils from central and northern California were very similar and were devoid of (E)- $\beta$ -ocimene and 6-methyl-5-octen-one. Considerable differentiation was found among populations of var. *concolor*, confirming the work of Zavarin et al. (1970, 1975) of the existence of the Cuyamaca Race, and three sub-types of *A. c.* var. *concolor* oils: group A (Utah), B1 (New Mexico) and B2 (Arizona). *Phytologia* 93(2): 208-220 (August 1, 2011).

**KEY WORDS:** *Abies concolor* var. *concolor*, *A. c.* var. *lowiana*, leaf essential oils composition, geographic variation.

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*Abies concolor* (Gord. & Glend.) Hilde. is a forest tree of western North America (Fig. 1) ranging from Oregon to northern Mexico (Zavarin et al. 1975). Eckenwalder (2009) recognized two varieties: var. *concolor* and var. *lowiana* (Gord.) Lemm. and noted that these have been treated as species by some authors. He also indicated that var. *lowiana* hybridizes with *A. grandis* (D. Dougl. ex D. Don in Lamb.) Lindl. but not with *A. lasiocarpa* (Hook.) Nutt. Recently, Xiang et al. (2009) examined nrDNA sequence data and found *A. concolor* most closely related to *A. grandis*, so hybridization seems possible. Zavarin et al. (1970, 1975) analyzed wood monoterpenes of *A. concolor* from 43 populations and found evidence that var. *lowiana* from n. and c. California formed a group (Fig. 1), but called var. *lowiana* from s. California, the Cuyamaca race. In addition, Zavarin et al. (1975) subdivided var. *concolor* into three groups (A, B1 and B2, Fig. 1).

There appears to be only one paper reporting on the leaf essential oil of *A. concolor* (Wagner et al. 1989) from a population in the North Kaibab Ranger District, AZ, and those data were reported on a ppm basis instead of the normal percent total oil data.

Previously (Adams et al, 2011) the volatile leaf oils of *Abies concolor* were reported to be highly differentiated in a Cimarron, NM population. Unfortunately, due to a sample numbering error, the results for the New Mexico population were incorrect. This paper reprints the original paper with corrected data.

## MATERIALS AND METHODS

Plant specimens: *Abies concolor* var. *concolor*: Adams 12405-12407, Mill B trailhead, Big Cottonwood Canyon, Salt Lake City, UT, 40° 37.996' N, 111° 43.418' W, 6242 ft., Adams 12481-12485, (by D. Thornburg) 7 mi. nw of Pine, AZ along Rim Rd., 34° 26.844'N, 111° 21.520'W, 7597 ft., Adams 12679-12683, 13 mi. w of Cimarron, NM on US 64, 36° 31.509' N, 105° 10.932' W, 7872 ft.

*Abies concolor* var. *lowiana*: Adams 12427-12431 (by R. Lanner) 2 mi. n of jct. US50 on White Meadows Rd., ca. 22 mi e of Placerville, CA, 38° 47' 00" N, 120° 29' 20" W, 3450 ft., Adams 12432-12436 (by R. Lanner) Mormon Emigrant Trail at jct. with Park Creek Rd., ca. 24 mi ese of Placerville, CA, 38° 43' 30" N, 120° 28' 20" W, 4000 ft., Adams 12438-12442 (by M. Kauffmann) Klamath Mtns., CA, 40° 50' 21.4" N, 123° 43' 11.09" W, 4820 ft., Adams 12464-12468 (by B. Miller) Lee Summit, CA on Hwy 70/89, 39° 52.674' N, 120° 45.736' W, 4414 ft., *Abies concolor* var. *concolor* / *lowiana*: Adams 12522-12526, on CA Hwy 38 north side of Onyx Summit, CA, 34° 12.037' N, 116° 43.520' W, 8490 ft. All specimens are deposited in the BAYLU herbarium.

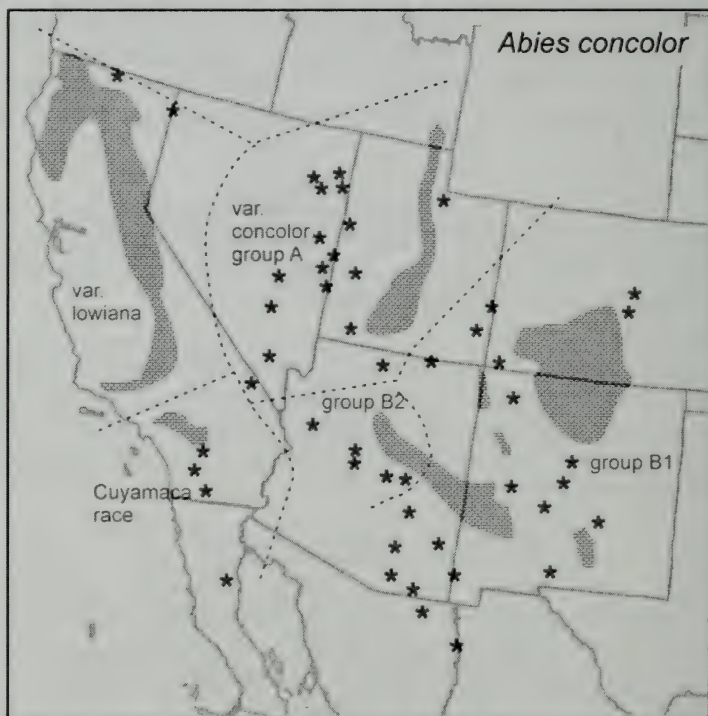


Figure 1. Distribution of *Abies concolor* (modified from Zavarin et al. 1975) with subgroups based on wood monoterpene data.



*Isolation of Oils* - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

*Chemical Analyses* - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

*Data Analysis* - Terpenoids (as percent total oil) were coded and compared among the species by the Gower (1971) metric. Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

## RESULTS AND DISCUSSION

In general, the leaf oils of *A. concolor* are dominated by monoterpenes with only small amounts of sesquiterpenes and diterpenes (Table 1). Each of the populations are high in  $\beta$ -pinene (Table 1). The populations from central and northern California (Lee S, Plac, Klam, Table 1) share several components at similar levels: linalool,  $\alpha$ -terpineol, geranyl acetate, RI 1617 sesquiterpene alcohol, and eudesm-7(11)-en-4-ol and all are devoid of (E)- $\beta$ -ocimene and 6-methyl-5-octen-one (Table 1).

The overall similarities of the oils are shown in figure 2. Notice *A. c.* var. *lowiana* from central and northwestern California have very similar oils (0.809, 0.888, Fig. 2). The major difference in the Klamath Mtns. oil is the presence of intermedeol that was only found in this oil and the New Mexico oil. The oils from Utah and Onyx Summit are the next most similar (0.734, Fig. 2), with the oils from Arizona and New Mexico being the least similar group (0.702, Fig. 2).

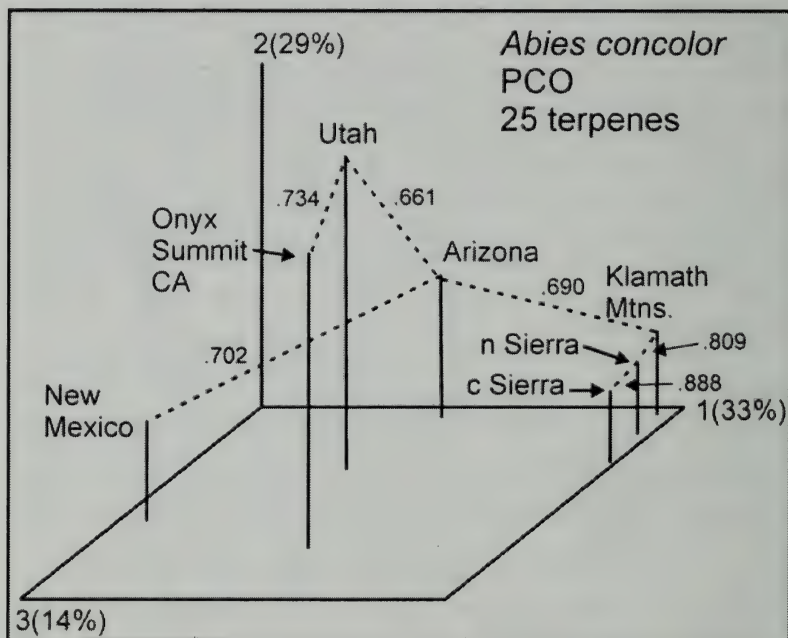


Figure 2. PCO based on 25 terpenes. The dotted lines are the minimum spanning network and the numbers next to the lines are the similarities.

Mapping the minimum spanning network onto the distribution of *Abies concolor* (Fig. 3) clearly shows the geographic affinities. The unity of the central and northwestern California *A. c.* var. *lowiana* populations is clear. Zavarin et al. (1975) designated the southern California populations as the Cuyamaca race and this analysis confirms their observation. Zavarin et al. (1975) found an affinity (in the wood monoterpenes) of the Cuyamaca Race to var. *concolor*, group A, and

there is a moderate similarity in their oils (Figs. 2, 3), but they are rather distinct.

Zavarin et al. (1975) divided *var. concolor* into 3 sub-groups: A, B1 and B2. The present analysis (based on leaf essential oils) confirms the same pattern of differentiation (Figs. 2, 3).

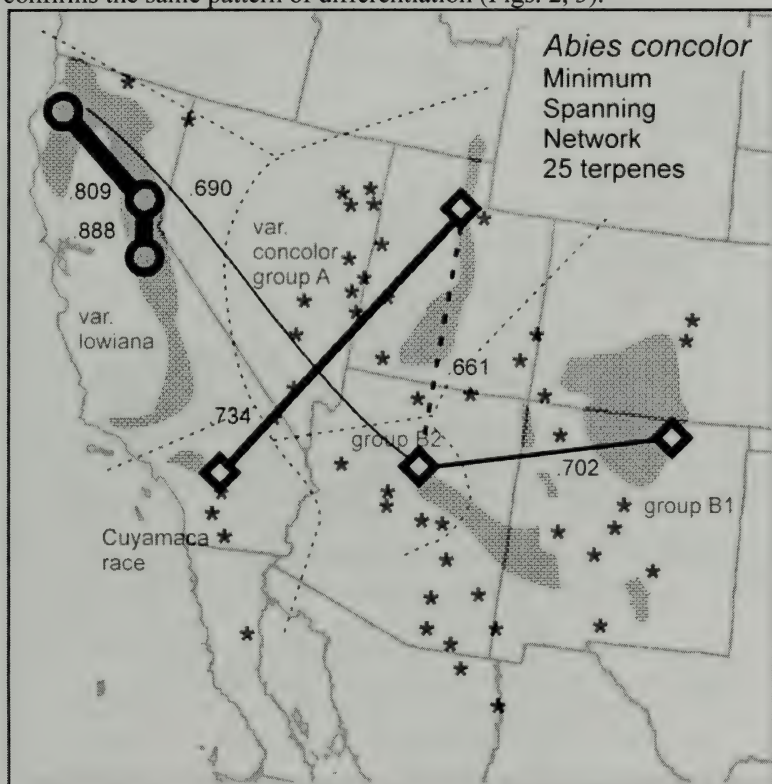


Figure 3. Minimum spanning network based on 20 terpenes. The open circles are *A. c. var. lowiana*, the open squares are generally treated as *A. c. var. concolor*. The numbers next to the lines are similarities.

A different perspective is obtained by geographically clustering the populations (Fig. 4). The differentiation of var. *lowiana* in northwest California is the dominant feature (Fig. 4). In addition, the differentiation of the other subgroups is clearly seen (Cuyamaca race - Utah, A) and (Arizona, B2 - New Mexico, B1).

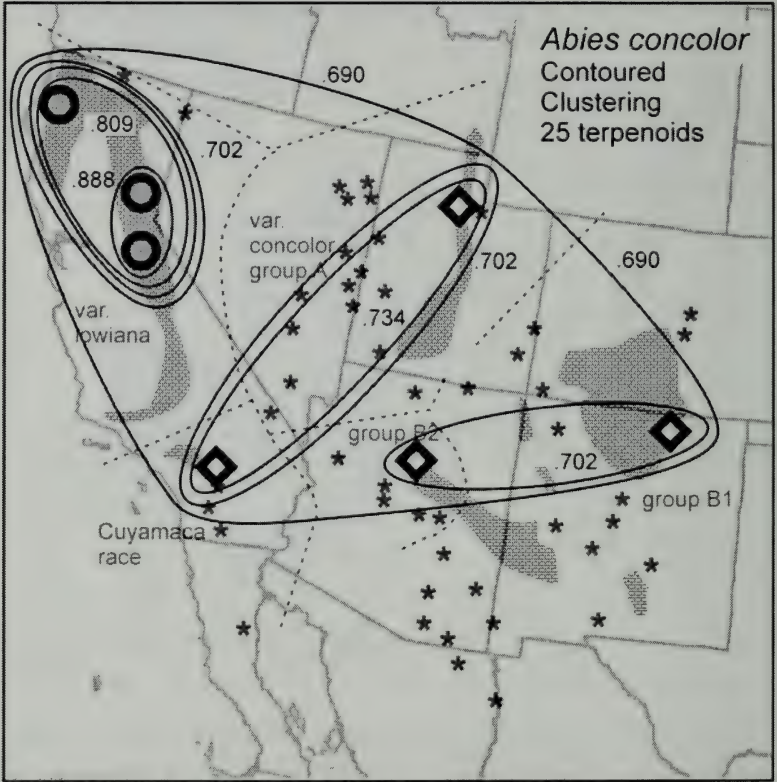


Figure 4. Geographically clustering of populations of *Abies concolor* based on 25 terpenoids. The numbers next to the contour lines are the similarities.



## CONCLUSIONS

In general, the leaf volatile oils gave a very good agreement with the pattern of differentiation Zavarin et al. (1975) found using wood monoterpenes. The volatile leaf oils support the recognition of var. *concolor* and var. *lowiana*. The differentiation of the Cuyamaca race, and three sub-types of *A. c.* var. *concolor* oils: group A (Utah), B1 (New Mexico) and B2 (Arizona) suggests that these populations might be incipient varieties.

## ACKNOWLEDGEMENTS

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Table 1. Comparison of leaf oil compositions of *Abies concolor* populations. Klam = Klamath Mtns., NW CA, Lee S = Lee Summit, CA, Plac = Placerville, CA, AZ = Pine, AZ, NM = Cimarron, New Mexico, UT = Wasatch Mtns., UT, Onyx = Onyx Summit, CA. Compounds in bold face appear to separate the taxa. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. RI is the Kovat's Index using a linear approximation on DB-5 column. \*= cpds used for PCO (30 cpds.)

RI	compound	Plac	Klam	Lee S	AZ	NM	UT	Onyx
884	<b>santene*</b>	<b>0.2</b>	<b>0.3</b>	<b>0.2</b>	-	-	<b>t</b>	<b>t</b>
921	tricyclene*	0.1	0.2	0.2	0.3	0.6	0.2	t
924	$\alpha$ -thujene	t	t	t	t	-	t	t
932	$\alpha$ -pinene*	4.4	4.7	5.1	7.9	6.6	8.9	7.8
946	<b>camphene*</b>	<b>1.4</b>	<b>2.7</b>	<b>2.5</b>	<b>4.3</b>	<b>9.0</b>	<b>2.9</b>	<b>0.5</b>
969	sabinene	t	t	t	t	0.1	t	t
974	<b><math>\beta</math>-pinene*</b>	<b>47.1</b>	<b>45.2</b>	<b>42.0</b>	<b>52.2</b>	<b>34.0</b>	<b>43.9</b>	<b>41.5</b>
988	myrcene*	2.2	1.4	2.0	2.2	1.2	2.1	1.7
1002	$\alpha$ -phellandrene	0.4	0.3	0.4	0.3	0.1	0.2	0.4
1008	$\delta$ -3-carene*	0.1	0.2	0.1	0.5	1.8	1.6	1.1
1014	$\alpha$ -terpinene	0.2	0.2	0.2	0.1	t	0.1	0.2
1020	p-cymene	t	t	t	t	t	t	t
1024	<b>limonene*</b>	<b>23.0</b>	<b>17.6</b>	<b>23.0</b>	<b>9.0</b>	<b>1.6</b>	<b>9.3</b>	<b>21.2</b>
1025	<b><math>\beta</math>-phellandrene</b>	<b>2.5</b>	<b>2.0</b>	<b>2.5</b>	<b>1.1</b>	<b>6.5</b>	<b>1.1</b>	<b>2.5</b>
1032	(Z)- $\beta$ -ocimene	-	t	t	0.3	0.1	0.2	0.1

RI	compound	Plac	Klam	Lee S	AZ	NM	UT	Onyx
1038	2-heptyl acetate	-	-	-	-	-	t	-
<b>1044</b>	<b>(E)-<math>\beta</math>-ocimene*</b>	-	-	-	<b>0.3</b>	<b>0.2</b>	<b>0.3</b>	<b>0.1</b>
1054	$\gamma$ -terpinene	0.2	0.1	0.2	0.2	0.2	0.2	0.2
1077	(6-methyl-5-octen-2-one)	-	-	-	0.1	0.1	0.1	0.1
1086	terpinolene*	1.7	1.0	1.4	0.7	0.6	1.7	1.4
1087	2-nonanone*	0.4	0.1	0.3	0.5	0.2	0.6	0.4
<b>1095</b>	<b>linalool*</b>	<b>0.2</b>	<b>0.1</b>	<b>0.4</b>	<b>1.4</b>	<b>1.9</b>	<b>1.8</b>	<b>1.4</b>
1118	endo-fenchol*	0.4	0.3	0.4	0.2	t	1.8	1.6
1118	cis-p-menth-2-en-1-ol	0.4	0.3	0.4	0.1	t	0.2	0.4
1122	$\alpha$ -campholenal	0.1	t	0.1	0.1	t	0.2	0.2
1136	trans-p-menth-2-en-1-ol	0.3	0.1	0.3	t	t	0.1	0.3
1141	camphor	t	t	0.1	t	0.2	0.1	t
<b>1145</b>	<b>camphene hydrate*</b>	<b>0.2</b>	<b>0.1</b>	<b>0.3</b>	<b>1.8</b>	<b>3.4</b>	<b>4.1</b>	<b>0.5</b>
1148	citronellal*	0.1	0.3	0.1	0.6	0.5	0.3	0.2
1155	iso-borneol	t	t	t	t	t	0.1	t
1165	borneol*	0.3	0.2	0.5	0.2	0.2	0.6	0.8
1172	cis-pinocamphone	t	t	t	t	t	-	t
1174	terpinen-4-ol*	0.4	0.3	0.4	0.3	0.4	0.6	0.7
1183	cryptone	t	t	t	t	-	-	t
<b>1186</b>	<b><math>\alpha</math>-terpineol*</b>	<b>6.9</b>	<b>4.8</b>	<b>6.5</b>	<b>2.2</b>	<b>1.2</b>	<b>3.5</b>	<b>4.5</b>
1195	cis-piperitol	0.2	0.1	0.2	t	t	t	0.1
1201	n-decanal	0.3	-	0.1	0.2	t	0.1	0.2
1207	trans-piperitol	0.1	t	0.2	t	-	t	0.1



RI	compound	Plac	Klam	Lee S	AZ	NM	UT	Onyx
1218	endo-fenchyl acetate	t	t	-	0.1	t	t	t
1223	citronellol*	0.2	0.3	0.5	0.4	0.2	0.6	1.0
1235	neral	0.1	-	0.3	-	-	t	t
1249	<b>piperitone*</b>	<b>0.1</b>	<b>t</b>	<b>0.4</b>	<b>t</b>	-	<b>1.1</b>	<b>1.7</b>
1264	geranial	0.4	-	0.6	t	t	0.2	0.2
1287	<b>bornyl acetate*</b>	<b>1.2</b>	<b>6.6</b>	<b>2.8</b>	<b>8.8</b>	<b>20.2</b>	<b>6.4</b>	<b>0.6</b>
1289	<b>thymol*</b>	<b>t</b>	-	<b>t</b>	<b>t</b>	-	<b>1.3</b>	<b>0.6</b>
1293	2-undecanone	0.3	0.2	0.1	0.4	0.3	0.1	0.4
1300	tridecane*	0.1	t	0.2	0.3	0.8	0.2	0.8
1350	citronellyl acetate*	0.2	0.2	0.2	0.5	0.2	t	0.1
1379	geranyl acetate*	0.8	0.4	0.5	0.2	0.6	t	t
1395	sesquiterpene, 43,55,86,206	0.1	-	0.1	0.3	t	t	-
1408	dodecanal	t	-	t	t	t	t	0.1
1417	(E)-caryophyllene	t	t	t	t	-	0.2	0.3
1478	$\gamma$ -muurolene	t	0.2	0.1	-	-	-	t
1496	valencene	0.2	0.3	0.2	t	-	-	0.2
1500	$\alpha$ -muurolene	-	-	-	-	t	-	-
1514	$\gamma$ -cadinene	-	-	-	-	0.3	-	-
1514	cubebol	-	0.2	-	0.1	0.3	-	0.3
1522	$\delta$ -cadinene	-	t	-	0.1	0.5	-	0.4
1559	germacrene B	t	-	0.1	t	-	-	t
1561	(E)-nerolidol	t	-	0.1	t	t	0.1	t

RI	compound	Plac	Klam	Lee S	AZ	NM	UT	Onyx
<b>1617</b>	<b>sesquiterpene, <u>81</u>, 161, 189, 222*</b>	<b>0.3</b>	<b>0.4</b>	<b>0.5</b>	<b>t</b>	<b>t</b>	<b>t</b>	-
1627	1-epi-cubenol	t	-	0.1	t	0.4	-	0.3
1649	$\beta$ -eudesmol	0.1	-	0.2	-	-	t	-
1652	$\alpha$ -eudesmol	0.1	-	0.2	-	-	t	-
1652	$\alpha$ -cadinol	0.1	-	0.2	-	t	t	-
<b>1665</b>	<b>intermedeol*</b>	-	<b>6.4</b>	-	-	<b>2.5</b>	-	-
1700	eudesm-7(11)-en-4-ol	0.1	0.1	0.1	-	-	t	-
1987	manoyl oxide	-	t	t	0.1	0.2	t	t
2014	palustradiene(=abieta-8, 13-diene) *	t	t	0.1	t	-	t	0.3
2056	manool	0.1	t	0.1	t	-	t	t
2149	abienol	0.1	t	0.1	t	-	t	0.2

**TAXONOMY OF INFRASPECIFIC TAXA OF *ABIES*  
*CONCOLOR* BASED ON DNA SEQUENCES FROM nrDNA AND  
FOUR CHLOROPLAST REGIONS**

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**ABSTRACT**

The DNA sequences from five gene regions (nrDNA, trnS-trnG, trnL-trnF, petN-psbM, psbM-trnD) for *Abies concolor* var. *concolor* and var. *lowiana* from seven populations from throughout their range were analyzed and sequences compared. Whereas the leaf essential oil compositions from these seven populations were quite differentiated, the DNA data revealed *A. concolor* to be fairly uniform having only 1-4 mutations (from 4556 bp of data) between populations, with the exception of the Klamath Mtns. population that was highly differentiated (in its trnS-trnG and psbM-trnD regions). The current DNA data does not support recognition of these taxa as distinct species nor do they support recognition of *A. concolor* var. *lowiana*. *Phytologia* 93(2):221-230 (August 1, 2011).

**KEY WORDS:** *Abies concolor* var. *concolor*, *A. c.* var. *lowiana*, DNA sequencing, SNPs, nrDNA, trnS-trnG, trnL-trnF, petN-psbM, psbM-trnD, Klamath Mtns., taxonomy.

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*Abies concolor* (Gord. & Glend.) Hilde. is a forest tree of western North America ranging from Oregon to northern Mexico (Zavarin et al. 1975). Hunt (1993) recognized both *A. concolor* and *A. lowiana*. Eckenwalder (2009) recognized two varieties: var. *concolor* and var. *lowiana* (Gord.) Lemm. and noted that these have been treated as species by some authors. Adams et al (2011) recently reported on the composition of the leaf essential oils and validated the chemical races reported by Zavarin et al. (1970, 1975); in general the leaf oils supported the recognition of vars. *concolor* and *lowiana* (Fig. 1).

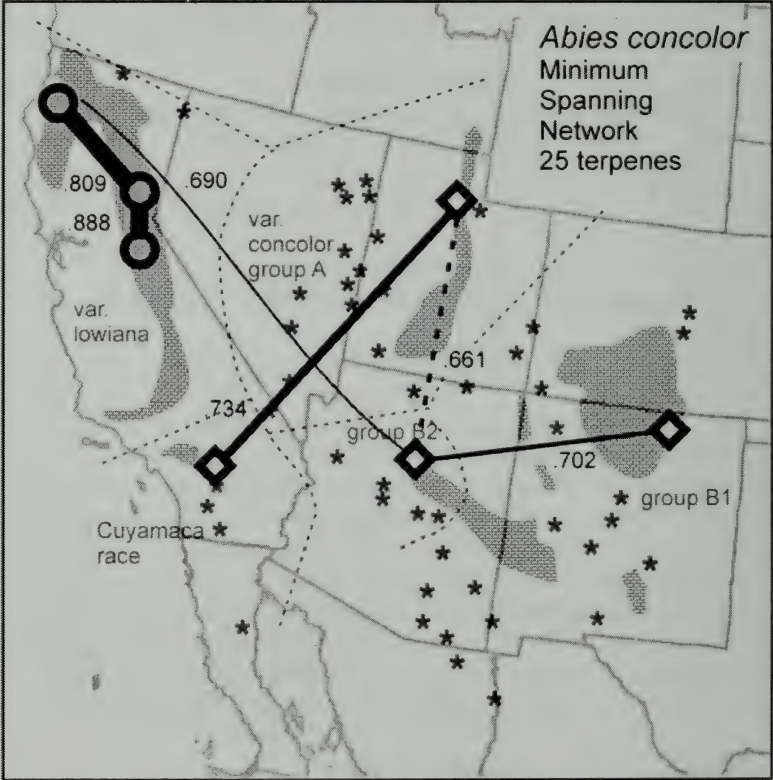


Figure 1. Minimum spanning network based on 20 terpenes of *A. concolor*. The open circles are *A. c. var. lowiana*, the open squares are generally treated as *A. c. var. concolor*. The numbers next to the lines are similarities. From Adams et al. (2011).



Xiang et al. (2009) recently published a phylogeny of *Abies* based on nrDNA sequences. They found *A. concolor* to form an unresolved clade with *A. grandis*, *A. religiosa* and *A. durangensis*. However, it is unlikely that nrDNA data alone is sufficient to portray phylogenetic relationships. In this study, we present sequence data for nrDNA, petN-psbM, psbM-trnD, trnL-trnF and trnS-trnD of *A. concolor* from throughout its range including the chemical races found by Zavarin et al. (1975) and Adams et al. (2011).

## MATERIALS AND METHODS

Plant specimens: *Abies concolor* var. *concolor*: Adams 12405-12407, Mill B trailhead, Big Cottonwood Canyon, Salt Lake City, UT, 40° 37.996' N, 111° 43.418' W, 6242 ft., Adams 12481-12485, (by D. Thornburg) 7 mi. nw of Pine, AZ along Rim Rd., 34° 26.844' N, 111° 21.520' W, 7597 ft., Adams 12679-12683, 13 mi. w of Cimarron, NM on US 64, 36° 31.509' N, 105° 10.932' W, 7872 ft.

*Abies concolor* var. *lowiana*: Adams 12427-12431 (by R. Lanner) 2 mi. n of jct. US50 on White Meadows Rd., ca. 22 mi e of Placerville, CA, 38° 47' 00" N, 120° 29' 20" W, 3450 ft., Adams 12432-12436 (by R. Lanner) Mormon Emigrant Trail at jct. with Park Creek Rd., ca. 24 mi ese of Placerville, CA, 38° 43' 30" N, 120° 28' 20" W, 4000 ft., Adams 12438-12442 (by M. Kauffmann) Klamath Mtns., CA, 40° 50' 21.4" N, 123° 43' 11.09" W, 4820 ft., Adams 12464-12468 (by B. Miller) Lee Summit, CA on Hwy 70/89, 39° 52.674' N, 120° 45.736' W, 4414 ft., *Abies concolor* var. *concolor* / *lowiana*: Adams 12522-12526, on CA Hwy 38 north side of Onyx Summit, CA, 34° 12.037' N, 116° 43.520' W, 8490 ft. Outgroup: *A. lasiocarpa* var. *bifolia*: Adams 12400-12404, Brighton Ski lodge parking lot. 40° 35' 48.76" N; 111° 35' 09.18" W, 2682m, Sept. 4, 2010, Salt Lake Co., UT. All specimens are deposited in the BAYLU herbarium.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

The nrDNA region of *Abies concolor* proved to be too large (~2000 bp) to sequence by use of ITSa and ITSb (Fig. 2).

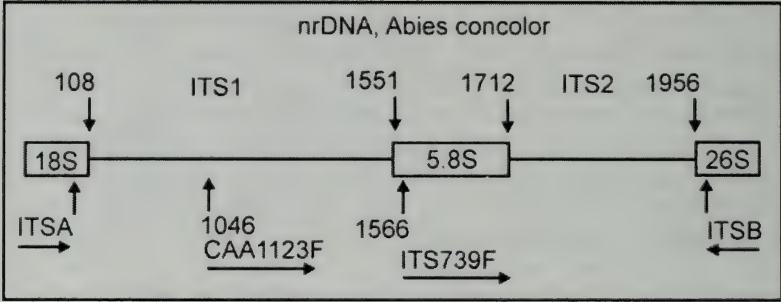


Figure 2. Diagram of the nrDNA region for *Abies lasiocarpa*.

Two addition primers were utilized:  
CAA1123F: AC CTC CTA TGT CGG TTG TGC (Xiang et al. 2009)  
ITS739F: AAC GGA TAT CTC GGC TCT, based on conserved sequences in the 5.8S region.

The trnC-trnD region of *Abies concolor* also proved to be large (~2400, Fig. 3). Due to the small area from trnC to petN, that region was skipped. Two regions were sequenced: petN-psbM and psbM-trnD using four primers (Fig. 2) based on sequences of *Abies* from GenBank:  
petNAc373F: TGG TAG TTT TTA CAT TTT CC,  
psbMAc1294R: TTA TCC CTT ACG TCA AAA CG  
and  
psbMAc1382F: AGA TCC ATG AAA TAG ATG TG  
trnDrev: GGG ATT GTA GTT CAA TTG GT

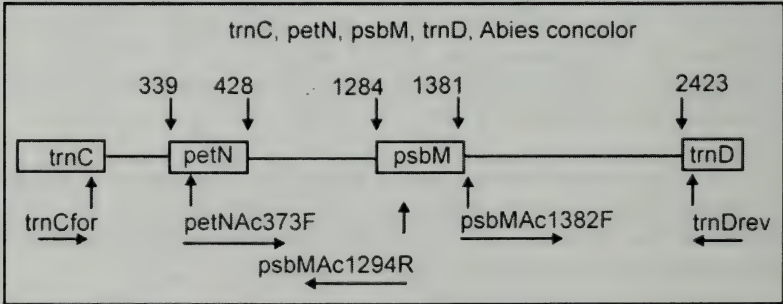


Figure 3. Diagram of the trnC-trnD region of *Abies concolor*.

Primers for trnL-trnF and trnS-trnG have been previously reported (Adams and Kauffmann, 2010).

PCR amplifications were performed in 30  $\mu$ l reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15  $\mu$ l 2x buffer E (cpDNA regions) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200  $\mu$ M each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM  $MgCl_2$  according to the buffer used) 1.8  $\mu$ M each primer. The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/>).

Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

## RESULTS AND DISCUSSION

Considering only the *Abies concolor* samples, different DNA regions gave variable numbers of taxonomically informative characters (Table 1). The nrDNA region gave no informative characters. The cpDNA regions proved the most useful, with psbM-trnD and trnS-trnG yielding 11 and 5 useful characters, respectively (Table 1).

Table 1. Summary of variation discovered in nrDNA, trnS-trnG, trnL-trnF, petN-psbM and psbM - trnD regions. The variation is based solely on *A. concolor* samples. #subs = # nucleotide substitutions.

region	bp	# subs	# indels	# single events	# informative characters (SNPs)
nrDNA	951	0	0	1	0
trnS-trnG	908	3	2	0	5
trnL-trnF	920	1	1	2	2
petN-psbM	824	2	0	1	2
psbM-trnD	953	5	6	2	11
totals	4556	11	9	6	20

Analysis of the concatenated five-gene sequences revealed unresolved clades of the Sierra and San Bernardino Mtns. populations (Figure 4). The major facet found was the uniqueness of the Klamath Mtns. population (Fig. 4) with a support of 97%. There is some support (69%) for the Arizona clade.

Examination of the 20 informative characters (Table 1), 11 are substitutions and 9 are indels. To utilize the information in the indels, these were coded as match/ mis-match data and a minimum spanning network was constructed. The minimum spanning network (Fig. 5) shows clearly the closeness of nearly all the *A. concolor* populations, except the Klamath Mtns. population, that differs by 16 mutational events from the Utah population.

To visualize the geographical variation among populations, a minimum spanning was plotted (Fig. 6) onto the chemical races map of Zavarin et al. (1975). Notice the broad links (highly related groups with few differences) linking var. *concolor* (open squares) and var. *lowiana* (open circles). The Klamath Mtns. individuals, although in relative close proximity to the Sierras populations, appear quite differentiated (Fig. 6).



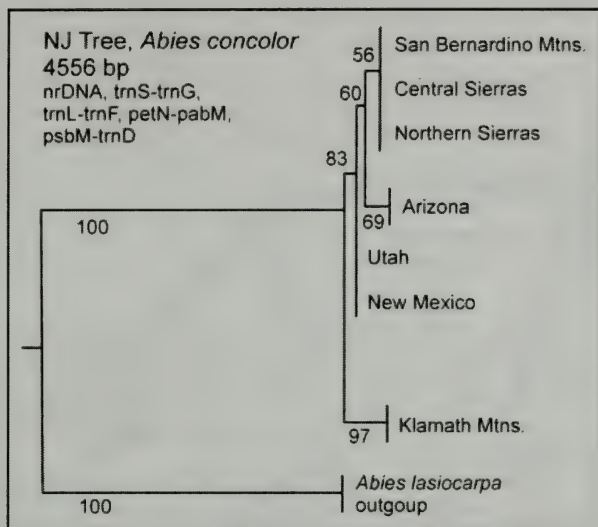


Figure 4. NT tree based on 4556 bp of sequence data. The numbers at the branch points are bootstrap values as percent.

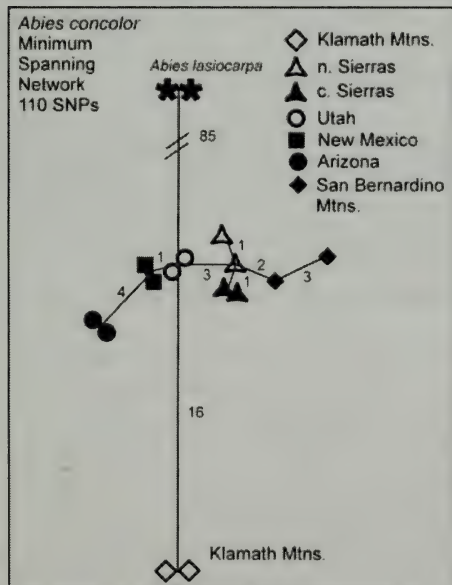


Figure 5. Minimum spanning network based on 110 SNPs. Numbers next to the line are the numbers of mutational events (substitutions + indels).

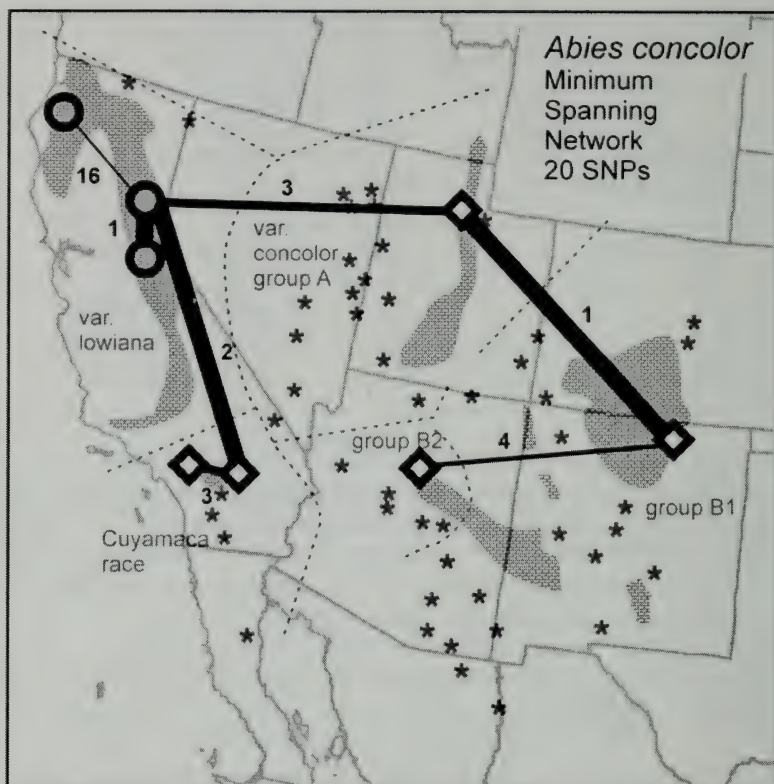


Figure 6. Minimum spanning network based on 20 SNPs, superimposed onto the chemical races map of Zavarin et al. (1975). The numbers next the links are the number of mutational events. The width of the links are inversely proportional to the relatedness.

It is interesting to compare the terpenoid differentiation (Fig. 1) with the DNA differentiation (Fig. 6). The DNA data shows basically one group for *A. concolor*, with an outlier from the Klamath Mtns. (Fig. 6) and no correlation with the chemical races of Zavarin et al. (1975) or Adams et al. (2011).

## CONCLUSIONS

Whereas the leaf essential oil compositions from seven populations were quite differentiated, the DNA data revealed *A. concolor* to be fairly uniform, having only 1-4 mutations (from 4556 bp of data) between populations, with the exception of the Klamath Mtns. population that was highly differentiated (for its trnS-trnG and psbM-trnD regions). It seems odd that only these two gene regions showed the differentiation of the Klamath Mtns. plants.

Hunt (1993) recognized both *A. concolor* and *A. lowiana*. The current DNA data gives no support for the recognition of these taxa as distinct species, nor support for the recognition of *A. concolor* var. *lowiana*.

Eckenwalder (p. 92, 2009) in writing about *A. concolor*, states that "It does, however hybridize and intergrade with the closely related grand fir (*A. grandis*) in northwestern California and southwestern Oregon". It may be that our samples of *A. concolor* from the Klamath Mtns. contain germplasm of *A. grandis*. This is currently being investigated.

## ACKNOWLEDGEMENTS

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**KEYS TO THE FLORA OF FLORIDA - 28,  
IRIS (IRIDACEAE)**

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**ABSTRACT**

*Iris* (Iridaceae) is represented in Florida by 8 species, one with two varieties. Most species are rare; none is endangered. All but one are native. *Iris savannarum* is endemic. *Iris pseudacorus* shows potential for becoming invasive. Differences between *I. brevicaulis*, *I. hexagona*, and *I. savannarum* are discussed. *Iris savannarum* var. *kimballiae* is recognized as a new combination and a lectotype is designated. A neotype for *I. brevicaulis* is selected. One species reported for Florida is excluded. An amplified key is given to the Florida taxa. *Phytologia* 93(2): 231-240 (August 1, 2011).

**KEY WORDS:** *Iris*, Iridaceae, Florida flora.

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By their beauty and ease of cultivation, species of *Iris* (Iridaceae) have long been popular in horticulture. A distinctive floral structure permits their immediate recognition to genus. But subtle, often hidden differences in their morphology have long contributed to misidentifications and misunderstandings among the species. The present study builds upon a substantial base of prior knowledge of the Florida irises. Yet for certain species there is still need for close reporting of their Florida distribution and clarification of floral structures.

Any mention of *Iris* in the Southeast immediately evokes recollection of an anomaly of American taxonomy: the designation of 98 "species" of iris (J. K. Small & E. J. Alexander, Contr. New York Bot. Gard. 327: 325-357. 1931; Small, Manual S.E. Flora. 1933) in an area where perhaps no more than a half-dozen species had previously

been acknowledged. Most of these finely differentiated entities were described from the Louisiana marshes. The type locality for many, near Kraemer, Lafourche Parish, was destroyed soon after their publication. But their presence in the literature has stimulated later workers either to discredit their existence or to make claims of similar hybrid swarms elsewhere. Publications attempting to clarify these many forms include: an assortment based upon their chromosomal numbers and forms (R. C. Foster, Contr. Gray Herb. 119: 3-82. 1937), an analysis of the separating characters (H. P. Riley, Amer. J. Bot. 25: 727-738. 1938; Amer. Iris Soc. Bul. 74: 3-7. 1939), and "negative" evidence of introgression among the species (L. F. Randolph, I. S. Nelson & R. L. Plaisted, Cornell Agric. Exp. Sta. Mem. 398: 3-56. 1967). These Louisiana-centered studies did not directly impact Florida botany except insofar as Florida species were represented. These are *I. brevicaulis* Raf., a Mississippi basin species whose range extends eastward to Florida, and *I. giganticaerulea* Small, a Louisiana endemic closely related to Florida's *I. savannarum* Small.

Magnificent color plates of five presumed species, two of them Florida endemics (*I. kimbballiae*, *I. savannarum*), were published by J. K. Small (Addisonia 9: 51-60. 1925; *ibid.* 12: 11-12. 1927). A floristic treatment of Florida species (as part of his report of all North American irises) has been prepared by N. C. Henderson (Fl. N. Amer. 26: 371-395. 2002).

Florida species of *Iris* fall within subgenus *Iris*, section *Pogiris*, subsection *Apogon*, series *Hexagonae* (*I. brevicaulis*, *I. fulva*, *I. hexagona*, *I. savannarum*), series *Laevigatae* (*I. pseudacorus*, *I. virginica*), series *Tripetalae* (*I. tridentata*), and series *Vernae* (*I. verna*), as classified by G. H. M. Lawrence (Gent. Herb. 8: 346-371. 1953). They may more usefully be divided into two groups by the form of their tricarpellate ovaries (and capsules). *Iris pseudacorus* L., *I. tridentata* Pursh, *I. verna* L., and *I. virginica* L. have carpels that remain externally visible and form 3 more or less distinct lobes or angles in the mature capsule, and rupture along a median suture in each carpel. Of these 3-lobed (*I. pseudacorus*) or 3-angled species, *Iris verna* occurs in dry woodlands; its habitat, and its slender cord-like rhizomes, are

distinctive. *Iris tridentata* is a plant of acid bogs, with unique one-flowered inflorescences and reduced petals. *Iris virginica* is marked by the several prominent veins in each blade and wholly herbaceous spathes.

*Iris pseudacorus*, the only introduced species, is the most robust -- and only yellow-flowered -- member of the Florida iris flora. Wherever it has become established it seems to spread inexorably by its sturdy rhizomes, forming dense stands that preclude other wetland species. Eradication efforts have been reported in retention ponds in Jackson County (Wildland Weeds 8: 12-13. 2005). But the few places where it is presently known have been insufficient to justify its formal ranking as an invasive species (fleppc.org, 2009).

*Iris fulva* Ker-Gawl., *I. hexagona* Walt., *I. brevicaulis* Raf., and *I. savannarum* Small bear two longitudinal flanges or ridges (faint, often absent in *I. savannarum*) on each carpel, which mature into a six-angled (or terete) capsule, and rupture irregularly by capsule wall disintegration. *Iris fulva*, by its bronze-red perianth, is outstanding and readily distinguished. It is of western origin; it has been found in Florida at only a single panhandle location. *Iris hexagona*, *I. savannarum*, and *I. brevicaulis* form a scarcely differentiated and poorly understood complex. *Iris hexagona*, the first described, was originally known in lowlands near the Santee River, South Carolina (Walter, 1788). Its outstanding character are the six prominent flanges on the ovary, maturing into a hexagonal capsule with six concave faces. Its present range is difficult to determine since herbarium materials are too often ambiguous. Henderson (2002: 392) reported *I. hexagona* to occur in Florida in only two counties of the northwest peninsula (a distribution confirmed here). He excluded *I. hexagona* from Georgia, thus implying a considerable disjunction from its South Carolina type locality. [Two apparently valid records from southern Georgia (S. B. Jones & N. C. Coile, 1988) seemingly were discounted.] H. H. Hume (Bull. Amer. Iris Soc. 1933) reported *I. hexagona* at three sites, now lost, in Nassau and Duval counties, northeastern Florida. *Iris hexagona* has been applied by some to include *I. savannarum*, a plant intended by its author (Small, 1925) to be restricted to the Florida peninsula, thus

confounding and vastly expanding the apparent distribution of *I. hexagona*.

*Iris brevicaulis* is a western species, common in the Louisiana marshes and northward, only sparingly reaching panhandle Florida. Its striking characteristic are flowering stems much shorter than the leaves, at times no more than 10-15 cm. long. Its capsules are very like *I. hexagona*.

*Iris savannarum* is by far the most abundant iris species in the state, once covering broad areas of Okeechobee prairie, now much reduced by agriculture and drainage but still common. It is the only species of the group without prominent flanges or ridges on the ovary and maturing into capsules circular in cross-section (if longitudinal ridges are present, they are low and inconspicuous, most often not sufficient to cause a cross-section to appear six-sided; but see var. *kimballiae*).

These three taxa show differences that taken as a whole justify specific rank for each. *Iris savannarum* inflorescences commonly overtop the leaves. Its ovaries show little or no longitudinal ribbings, and the capsules are essentially circular in cross-section. *Iris hexagona* inflorescences are usually somewhat shorter than the longest leaves. Its ovaries are prominently ridged longitudinally, and mature into capsules that are sharply hexagonal with each of the six faces concave. *Iris brevicaulis* inflorescences are very much shorter than the leaves with abrupt zigzags at each node. Its ovaries are also ridged and mature into hexagonal capsules.

Habitats also differ. *Iris savannarum* is usually found in standing water or on soils that are often fully saturated. *Iris hexagona*, at least in Florida, occurs on ditchbanks and road shoulders, seasonally wet but not long immersed. And *Iris brevicaulis* is often a plant of moist pastures or woodlands, apparently never flooded.

Botanists have differed in their treatment of these taxa. Small (1925, 1933), the author of *I. savannarum*, was confident they were



distinct species; he was followed in this belief by contemporaries whose interest was perhaps more in their horticulture and distribution (e.g., Hume, 1933). More recent writers (e.g., W. J. Dress, Hortus III. 1976), as well as the present authority (Henderson, 2002), also accepted *I. hexagona*, *I. brevicaulis*, and *I. savannarum* as species. Foster (1937) formed *I. hexagona* var. *savannarum*, though this new combination seems not to have gained traction. Others (e.g. R. P. Wunderlin, Guide to the Vasc. Plants of Florida, 1998) went a step further, recognizing *I. brevicaulis* and *I. hexagona*, with *I. savannarum* wholly submerged in the latter taxon.

Two names employed in Florida irises -- *I. rivularis* Small (1927), and *I. kimballiae* Small (1925) -- have resisted understanding. *Iris rivularis*, though splendidly illustrated and carefully described, appears to have evaded collection subsequent to its discovery in 1927 (cf. Hume, 1933). Small stated it to occur along streams flowing into the St. Mary's River, the divide between Georgia and northeastern Florida. Its morphology does not differ markedly from that of *I. hexagona* which has been reported in the same area (Hume), and it seems best to treat it as a tentative synonym of that species.

*Iris Kimballiae* presents another, presently unresolved problem. This taxon was given specific rank with its type locality the Apalachicola River delta, in the central panhandle. [Small originally (1925) reported this from both Apalachicola and northeast Florida, but later (1933) restricted it to the western station, the northeastern plants becoming his *I. rivularis*.] Small, on a July trip (J. N.Y. Bot. Gard. 31: 272-277. 1930), collected "ripe perfect capsules" of his *I. Kimballiae* for the "first time" (thereby giving credence to his description (1933) of them as "ellipsoid or oval, 5-9 cm. long, bluntly 6-sided"). Soon after, Hume (1933) on a March trip found flowering *I. Kimballiae* at eight locations near the town of Apalachicola. Foster (1937) also knew the plant; he excluded *I. fulva* as a possible parent, and reported its chromosomes to be like *I. brevicaulis* and *I. giganteaerulea* (of Louisiana) but with  $2n=42$  rather than their  $2n=44$ .

Hume's description of habitat and exact locations of *I. Kimballiae* and Foster's chromosome counts constitute the great bulk of

what is known of this taxon. Later authors, even though in some cases based not far from its type locality, seem wholly unfamiliar with it, either placing the name in synonymy under *I. hexagona* (Wunderlin, 1998; Wunderlin & Hansen, 2003) or disregarding it entirely (Godfrey & Wooten, 1979; Clewell, 1985). Since Small's description (especially its capsule) is in closer accord with *I. savannarum* than with other possible allies, lacking further knowledge, placement of *I. kimballiae* under *I. savannarum* seems justified. But its morphology (as well as its range) demonstrates differences from that species; varietal rank is indicated and a new combination is required.

/ *Iris savannarum* J. K. Small var. *kimballiae* (J. K. Small) D. B. Ward, comb. et stat. nov. Basionym: *Iris Kimballiae* J. K. Small, Addisonia 9: 59-60, plate 318. 1925. TYPE: U.S.A. Florida: Franklin Co., swamp, Apalachicola, 1921. Specimens prepared June 1923. Three-sheet LECTOTYPE (*Small* 49912, 49917, 49918 - NY) designated here.

Typification of *Iris Kimballiae* was handled somewhat irregularly. Best as can be determined, in 1921 Winifred Kimball, resident of Apalachicola, sent living material to Small, which was placed in the NY "propagation house." In June 1923, Mary E. Eaton, from flowering material, painted the illustration later published in Addisonia. Apparently also in 1923, three specimens were prepared from the living plant and were labeled by Small as from Kimball and "Swamp, Apalachicola, Fla." Small (his handwriting) assigned them his collection numbers 49912, 49917, and 49918. In July 1924, Small visited the Apalachicola site and made two collections (NY). At publication of *I. Kimballiae* (Addisonia, 1925), Small remarked that "type specimens...are in the [NY] herbarium." In 1985 A. F. Cholewa (NY) annotated the three Kimball specimens as syntypes and noted that Small had not indicated which was to be the type. Small's original material thus consisted of three specimens from greenhouse materials, two collections from Apalachicola, and the color plate published in conjunction with his new name and protologue. Here, the three specimens prepared from the Kimball plant are considered a three-sheet lectotype.

A detail of typification remains unaddressed. Foster (1937) apparently was the first author to equate the previously overlooked *Iris brevicaulis* Raf. (*Florula Ludoviciana*, 20. 1817) with the formerly widely used *I. foliosa* Mack. & Bush (*Trans. Acad. Sci. St. Louis* 12: 80-81. 1902). Foster noted the similarity of the two descriptions left "no doubt" that Rafinesque and MacKenzie & Bush were addressing the same plant. But there was still room for uncertainty; Rafinesque had prepared his *Florula* by editing and translating (from the French) a detailed but amateurish description of Louisiana plants by C. C. Robin (1807). Since Rafinesque never saw whatever specimens Robin may have had, his names are without types. To remove doubt as to the form represented by Robin's plant, and to avoid any possibility of conflict between the two names, the type for *I. brevicaulis* selected here is the same specimen as that designated by MacKenzie & Bush for their *I. foliosa*.

*Iris brevicaulis* Rafinesque, *Florula Ludoviciana*, p. 20. 1817. NEOTYPE, selected here: K. K. MacKenzie & B. F. Bush *s.n.*, 6 June 1897 (MO), Little Blue Tank, Jackson Co., Missouri, U.S.A. This is also an isotype of *Iris foliosa* Mack. & Bush, *Trans. Acad. Sci. Soc. St. Louis* 12: 80-81. 1902. ["Little Blue Tank" was a steam-locomotive water-supply tank at railroad crossing near Little Blue Spring, s.e. edge of Independence, Mo.]

## IRIS L.      Irises, Flags<sup>1</sup>

1. Flowers yellow; central mark of sepals sharply margined; capsules strongly 3-lobed in x-section, each lobe with groove along crest; plants tall. Perennial herb, to 2 m. Open marshes, wooded sloughs. Western and central panhandle (Escambia, Jackson, Leon counties), northern peninsula (Alachua Co.); rare. Spring. Appearing invasive, but not yet so classified.

YELLOW IRIS.

\* *Iris pseudacorus* L.

1. Flowers reddish brown or blue to purple; central mark of sepals shading into blade; capsules  $\pm$  3-angled (*I. verna*, *I. tridentata*, *I. virginica*), or terete to 6-angled in x-section.

2. Flowers coppery red to bronze; petals much shorter than claw of the sepals; capsules 6-angled. Perennial herb, to 1 m. Shallow water of riverside swamp. Western panhandle (Santa Rosa Co.); rare. Spring.

COPPER IRIS.

***Iris fulva* Ker-Gawl.**

2. Flowers blue to purple (rarely white); petals equal to or longer than claw of the sepals.

3. Plants small, the leaves <15 cm. long, 0.5-1.0 cm. broad; rhizomes slender, cord-like; perianth fused basally into a long (3-5 cm.) slender tube; petals violet; sepals violet with papillose yellow central band; xeric. Perennial herb, to 0.2 m. Dry sandy woodlands. Western panhandle (Escambia, Santa Rosa counties); rare. Spring. [*Neubeckia verna* (L.) Alef.]

DWARF IRIS.

***Iris verna* L. var. *smalliana* Fern.**

3. Plants larger, the leaves 20-50 cm. long; rhizomes stout; perianth fused basally into a very short (<1 cm.) tube; mesic to hydric.

4. Petals small and inconspicuous, scarcely longer than claws of the sepals; inflorescence usually one-flowered. Perennial herb, to 0.6 m. Seepage bogs. Central panhandle (Bay to Wakulla counties), disjunct east to northeast Florida (Duval Co.); rare. Spring. [*Iris tripetala* Walt.] ***Iris tridentata* Pursh**

4. Petals apparent, at least  $\frac{2}{3}$  as long as the full sepal; inflorescence usually several-flowered.

5. Leaves with 1-3 prominent nerve-like longitudinal veins; peduncle extended beyond subtending spathes; capsules somewhat 3-angled; spathes (both inner and outer) wholly herbaceous. Perennial herb, to 0.8 m. Marshes, ditches, river floodplains. Central panhandle (Washington Co.), east across north Florida (to Nassau, Duval counties), south to coastal upper peninsula (Taylor, St. Johns counties); rare. Spring.

BLUE FLAG, BLUE IRIS..

***Iris virginica* L.**



5. Leaves with all veins of similar prominence; peduncle enclosed within subtending spathes; capsules 6-angled to terete; spathes herbaceous to scarious (the inner often wholly scarious).
6. Ovaries at anthesis circular in x-section, without prominent longitudinal flanges or ridges (or with inconspicuous ridges in var. *kimballiae*); mature capsules mostly >5 cm. long, circular in x-section (or with each of the 3 carpels slightly tumid); leaves usually overtopped by inflorescences. Perennial herb, to 1 m. Spring-summer. Endemic.

PRAIRIE IRIS.

**Iris savannarum** Small

- a. Mature capsules terete, with or without 6 low longitudinal ridges; petals and sepals narrowly spatulate. Savannas, marshes. Peninsula (n. to Levy, Alachua counties); common (often locally abundant in mid-peninsula, rare or absent in s.e. coast, absent from Keys). [*Iris Albispiritus* Small; *Iris hexagona* var. *savannarum* (Small) Foster]

PRAIRIE IRIS (type).

var. **savannarum**

- a. Mature capsules 6-sided; petals and sepals broadly spatulate. Marshy stream banks, sometimes brackish. Coastal mid-panhandle (Franklin Co.); rare. [*Iris Kimballiae* Small]

KIMBALL IRIS. var. **kimballiae** (Small) D. B. Ward

6. Ovaries at anthesis circular in x-section but with each of the 3 carpels bearing two longitudinal flanges or ridges (thus pistil with 6 equal-spaced flanges); mature capsules mostly <6 cm. long, hexagonal in x-section with each face concave (each face corresponding to surface between the 6 former flanges); leaves usually overtopping inflorescences.
7. Flowering stem 60-100 cm. tall, scarcely zigzag, erect, shorter than to nearly equal the basal leaves; usually with single flowers opening in sequence. Perennial herb, to 1 m. Open marshes, ditch banks. Upper western peninsula (Taylor, Dixie counties); rare. Spring. Far-disjunct from its type locality in coastal South Carolina. [*Iris rivularis* Small (?)]

WALTER'S IRIS.

**Iris hexagona** Walt.

7. Flowering stem 20-40 cm. tall, sharply zigzag (alternately flexed at each node), often declining, much shorter than and partly hidden by the basal leaves, often with several simultaneous flowers. Perennial herb, to 0.8 m. Open mesic woodlands. Central panhandle (Gadsden, Jackson counties); rare. Spring. [*Iris foliosa* Mack. & Bush.]

ZIGZAG IRIS.

*Iris brevicaulis* Raf.

Excluded names:

***Iris germanica* L.**

Reported for Jackson Co. (Anderson, 1989; Godfrey 80395 - FLAS, FSU). Not confirmed to be naturalized.

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1. This paper is a continuation of a series begun in 1977. The "amplified key" format employed here is designed to present in compact form the basic morphological framework of a conventional dichotomous key, as well as data on habitat, range, and frequency. Amplified keys are being prepared for all genera of the Florida vascular flora; the present series is restricted to genera where a new combination is required or a special situation merits extended discussion.

I have been helped in the field by Christine M. Hausel, Robert T. Ing, and Robert W. Simons, and with records from the panhandle by Loran C. Anderson. I am especially indebted to John Beckner for making available his years of observation of the native species of *Iris*.

**A NEW SPECIES OF *SCUTELLARIA* (LAMIACEAE)  
FROM OAXACA, MEXICO**

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**ABSTRACT**

***Scutellaria serboana*** B.L. Turner, **sp. nov.** is described from Oaxaca, Mexico. It belongs to the previously monotypic Sect. *Crassipedes*, where it nestles easily next to *S. hintoniana* Epling, the latter known only from the state of Mexico. A photograph of the type is provided, along with a map showing its distribution. *Phytologia* 93(2): 241-244 (August 1, 2011)

**KEY WORDS:** *Scutellaria*, Lamiaceae, Mexico, Oaxaca

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Routine identification of Oaxacan Lamiaceae has occasioned the present paper.

**SCUTELLARIA SERBOANA** B.L. Turner, **sp. nov.** Fig. 1

*Scutellariae hintonianae* Epling similes sed differt floribus minoribus (corollas 20-25 mm longis vs 33-34 mm; calycibus 3-4 mm longis, vs ca 6 mm) et petiolis midcaulinibus multo longioribus (15-30 mm longis vs 3-15 mm).

**TYPE SPECIMEN: MEXICO. OAXACA: Mpio. San Miguel del Puerto**, Cerro el Vigia, "Selva mediana subperennifolia. suelo negro." ca 1671 m, 16 00 50.4 N, 96 06 46.2 W, 9 Aug 2006, *Jose Pascual* 1947 (Holotype: TEX).

**Perennial herbs**, to 50 cm high. **Mid-stems** pubescent with mostly minute, recurved hairs. **Leaves** (upper), 4-9 cm long, 2-4 cm wide; petioles, 1.5-3.0 cm long, pubescent like the stems; blades, ovate,

glabrous above and below, or nearly so, the margins irregularly crenulate. **Capitulescence** a short terminal raceme of 4-6 paired flowers, the axis glandular-pubescent, bracteate with persistent ovate bracts 2-3 mm long. **Pedicels** 3-4 mm long. **Calyces** (flowering) 3-4 mm long, pubescent with both glandular and non-glandular, recurved hairs. **Corollas** reportedly "guinda," 20-25 mm long, moderately pubescent externally, upper lip ca 5 mm long, lower ca 2 mm long, the tube ca 23 mm long, markedly flared upwards, ca 8 mm wide at the orifices. **Stamens** 4, scarcely exerted, if at all, the upper anthers ca 1 mm long. **Nutlets** not observed.

The species name is an acronym of the Sociedad para el Estudio de los Recursos Bioticos de Oaxaca (SERBO). This organization has helped fund the collection of numerous plants from the area concerned.

The novelty apparently belongs to the previously monotypic Sect. *Crassipedes*, as delimited by Epling (1939). Paton (1990), after a cosmopolitan study, redefined *Scutellaria*, positioning *S. hintoniana* within his broad concept of Sect. *Scutellaria*, treating this as the only member of his "*S. hintoniana* species-group" (13), noting its relationship to the "*S. caerulea* species-group" (16).

### ACKNOWLEDGEMENTS

My colleague, Guy Nesom, provided the Latin diagnosis, and reviewed the paper, for which I am grateful. The distribution map (Fig. 2) is based upon specimens on file at TEX.

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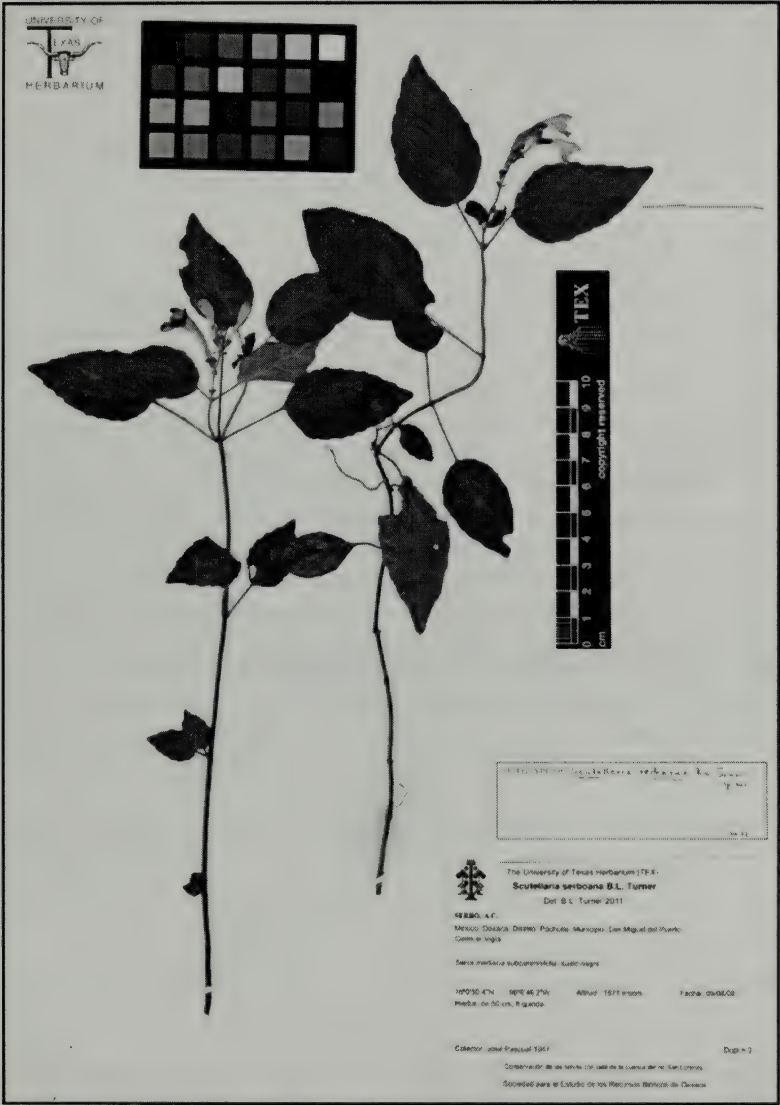


Fig. 1. *Scutellaria serboana* (Holotype: TEX).

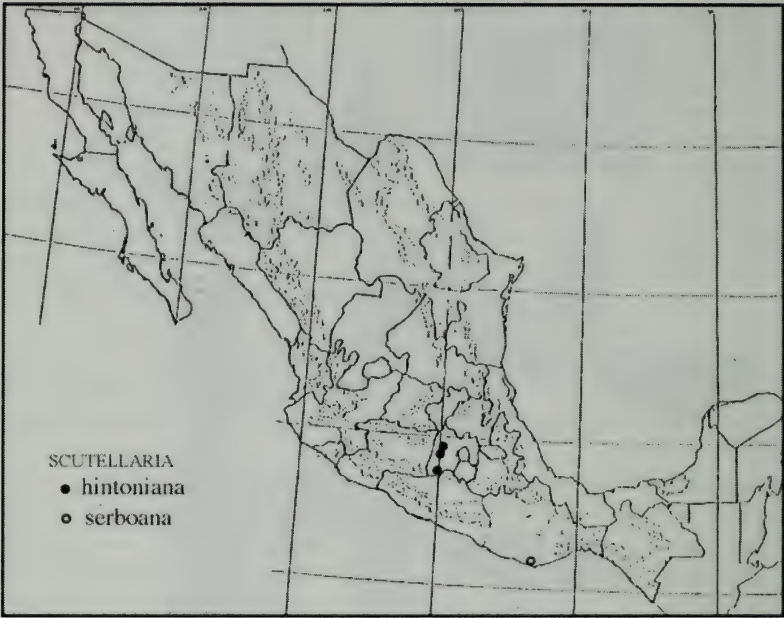


Fig. 2. Distribution of *Scutellaria hintoniana* and *S. serboana*.

## GEOGRAPHIC VARIATION IN THE LEAF ESSENTIAL OILS OF *JUNIPERUS CALIFORNICA*

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### ABSTRACT

The volatile leaf oils of *Juniperus californica* were analyzed from throughout the species range in the United States. Three groups of *J. californica* were found: the Central Valley, the deserts of southern California, and a northwestern Arizona group. The oils of the Central Valley populations were very uniform and very low in  $\alpha$ -pinene, with a moderate amount of sabinene, and high in camphor. Their oils contain 8 diterpenes not found in other *J. californica* populations. The oils from the southern California desert populations ranged from Vasek and Scora's 'Cal A' oil [high in  $\alpha$ -pinene (30.3%), sabinene (19.3%) and low in camphor (5.8%)] to 'Cal B' oil [moderate amounts of  $\alpha$ -pinene and sabinene and a high concentration of camphor (21.9%)]. However, the chemical races of Vasek and Scora (1967) were found as a mosaic that did not fit any geographic pattern in southern California. The differentiation of the Central Valley populations appears to be due to a post-Pleistocene migration from germplasm in the southern California deserts. *Phytologia* 93(2): 245-259 (August 1, 2011).

**KEY WORDS:** *Juniperus californica*, leaf essential oils composition, geographic variation.

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Vasek and Scora (1967) presented preliminary analysis of the leaf essential oils of *Juniperus californica* and suggested that there were two chemical races (Cal A and B). Adams et al. (1983) re-analyzed the leaf oils of the same populations that Vasek and Scora (1967) studied and found that Cal A oil was high in sabinene,  $\beta$ -pinene, camphor and terpinen-4-ol, whereas these compounds were low in Cal B. In contrast,  $\alpha$ -pinene was found to be high in Cal B oil and low in Cal A. Adams (2000, 2011) further characterized the oils of these chemical types.

To date, no comprehensive geographic study of the leaf essential oil of *J. californica* has been published. The purpose of the present paper is report on geographic variation in the leaf essential oil of *J. californica* and to attempt to clarify the purported chemical races, A and B.

MATERIALS AND METHODS

Plant specimens (populations shown in Figure 1): *Juniperus*

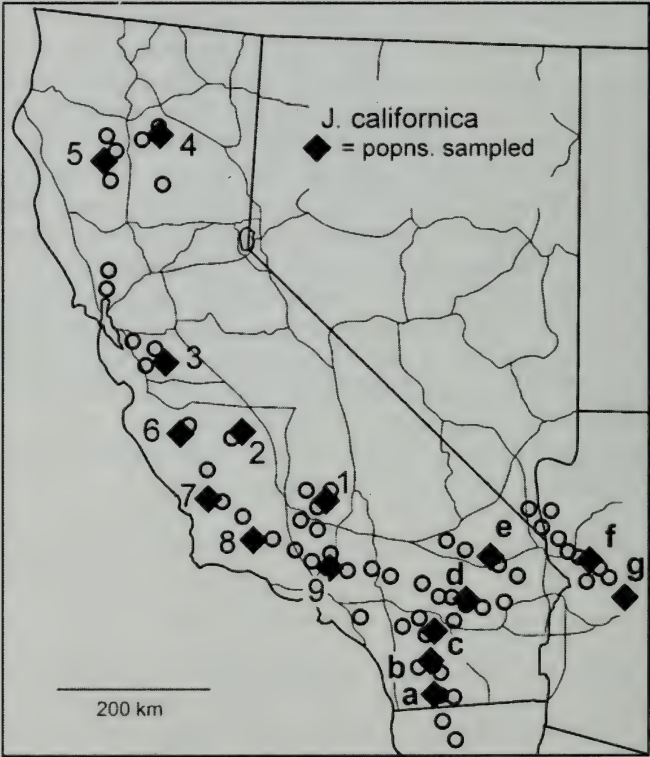


Figure 1. Distribution of *J. californica* (based on Vasek, 1966) and populations sampled (black quadrangles).



*californica*: Popn. 1, *Adams 12145-49*, Bodfish, CA; Popn. 1, *Adams 12145-49*, 4.8 mi. s of Bodfish, CA on CA483, Lat.  $35^{\circ} 33.252'$  N; Long.  $118^{\circ} 30.385'$  W, 1023 m; Popn. 2, *Adams 12150-54*, 8 mi. SW of Coalinga, CA on CA198, ca. 20 mi. w of hwy I5, Lat.  $36^{\circ} 05.762'$  N; Long.  $120^{\circ} 27.245'$  W, 315 m; Popn. 3, *Adams 12155-12159*, on Del Puerto Canyon Rd., 12 mi w of hwy. I5. Lat.  $37^{\circ} 26.186'$  N; Long.  $122^{\circ} 19.494'$  W, 256 m, Del Puerto Canyon, CA; Popn. 4, *Adams 12160-12164*, 8 mi ne of Red Bluff, CA on CA 36, Lat.  $40^{\circ} 17.066'$  N; Long.  $122^{\circ} 07.006'$  W, 272 m; Popn. 5, *Adams 12165-12169*, 4 mi sw of Lakeport, CA, Lat.  $38^{\circ} 59.709'$  N; Long.  $122^{\circ} 55.802'$  W, Elev. 424 m; Popn. 6, *Adams 12170-12174*, 3.5 mi. e on CA146 at west entrance to Pinnacles Natl. Park, CA, Lat.  $36^{\circ} 28.417'$  N; Long.  $121^{\circ} 13.513'$  W, 605 m, Popn. 7, *Adams 12175-12179*, 19 mi. w. of US101, 17 mi e of Santa Margarita, CA, Lat.  $35^{\circ} 28.137'$  N; Long.  $120^{\circ} 22.753'$  W, Elev. 450 m, Popn. 8, *Adams 12180-12184*, on CA33, 12 mi s of jct of CA33 and CA166, ~25 mi sw of Maricopa, CA, Lat.  $34^{\circ} 46.010'$  N; Long.  $119^{\circ} 25.241'$  W. Elev. 981 m; Popn. 9, *Adams 12185-12189*, on CA N2, ~2mi w of Palmdale, CA, Lat.  $34^{\circ} 35.007'$  N; Long.  $118^{\circ} 10.489'$  W. Elev. 844 m; Popn. a, *Adams 12190-12194*, on hwy I8, mile 76. 11 mi. sw of Ocotillo, CA, Lat.  $32^{\circ} 38.175'$  N; Long.  $116^{\circ} 07.103'$  W, Elev. 989 m; Popn. b, *Adams 12195-12199*, on CA S2, 12-15 mi s of Scissors Crossing, CA, Lat.  $33^{\circ} 01.053'$  N; Long.  $116^{\circ} 25.789'$  W. Elev. 801 m; Popn. c, *Adams 12200-12204*, on CA 74, Pinyon Flats campground. ~10 mi sw of Palm Desert. Lat.  $33^{\circ} 34.981'$  N; Long.  $116^{\circ} 27.383'$  W. Elev. 1228m; Popn. d, *Adams 12205-12209*, on CA 62, 1.5 mi s of Yucca Valley City center, Lat.  $34^{\circ} 06.724'$  N; Long.  $116^{\circ} 28.361'$  W. Elev. 1044 m; Popn. e, *Adams 5067-5071*, 8.0 mi. N of I40 on Rd to Kelso, CA at Microwave Station,  $34^{\circ} 48' 40.24''$  N, Long.  $115^{\circ} 36' 32.62''$  W, Elev. 1300 m; Popn. f, *Adams 5072-5076*, 17 mi se of Yucca, AZ, on road to Alamo Lake, AZ,  $34^{\circ} 42' 53''$  n,  $113^{\circ} 54' 49''$  w, 950 m; Popn. g, *Adams 12117-12121*, 5 mi. NW of Jct. of AZ97 and US93 on w side of US93, 2 mi. se of Mohave/ Yavapai Co. line, Lat.  $34.46695^{\circ}$  N; Long.  $113.31133^{\circ}$  W, Elev. 987 m. All specimens are deposited in the BAYLU herbarium.

A summary of the populations sampled is given in Table 1.

Table 1. Summary of populations sampled.

Popn.	Location	Lat/ Long	Elev.
Central Valley			
1	Bodfish	35° 33.252' N 118° 30.385' W	1023 m
2	Coalinga	36° 05.762' N 120° 27.245' W	315 m
3	Del Puerto Canyon	37° 26.186' N 122° 19.494' W	256 m
4	Red Bluff	40° 17.066' N 122° 07.006' W	272 m
5	Lakeport	38° 59.709' N 122° 55.802' W	424 m
6	Pinnacles Natl. Park	36° 28.417' N 121° 13.513' W	605 m
7	Santa Margarita	35° 28.137' N 120° 22.753' W	450 m
8	sw of Maricopa	34° 46.010' N 119° 25.241' W	981 m
9	Palmdale	34° 35.007' N 118° 10.489' W	844 m
southern California desert			
a	sw of Ocotillo	32° 38.175' N 116° 07.103' W	989 m
b	Scissors Crossing	33° 01.053' N 116° 25.789' W	801 m
c	Pinyon Flats CG	33° 34.981' N 116° 27.383' W	1228 m
d	Cal B' Yucca Valley City	34° 06.724' N 116° 28.361' W	1044 m
e	Cal A' s of Kelso	34° 48.671' N 115° 36.544' W	1300 m
northwestern Arizona			
f	se of Yucca, AZ	34° 42.883' N, 113° 54.817' W	950 m
g	2 mi. se of Mohave/ Yavapai Co. line, AZ	34° 28.017' N 113° 18.680' W	987 m

*Isolation of Oils* - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

*Chemical Analyses* - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic

reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as percent total oil) were coded and compared among the species by the Gower (1971) metric. Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

## RESULTS AND DISCUSSION

The compositions of leaf oils from various populations are listed in Table 2. All the components (>0.5% total oil) are now identified, except two diterpenes. The Central Valley populations (Bod, RBf, Lkp) are each very low in  $\alpha$ -pinene, have a moderate amount of sabinene, and are high in camphor (Table 2). But the most characteristic components are the diterpenes: rosa-5,15-diene (ent-), pimaradiene, unknown diterpene, KI 1973, manoyl oxide, geranyl linalool (E,E), abietatriene, sandaracopimarinal, sandaracopimarinal, trans-totarol and trans-ferruginol (Table 2). Most of these are only present in the Central Valley populations. All the Central Valley populations except Palmdale (on the very southernmost end) were very uniform among tree oils. In contrast, the oils of all the southern California desert and Arizona populations were very variable. The oil of the Kelso population (Kel) was called 'Cal A' by Vasek and Scora (1967) was high in  $\alpha$ -pinene (30.3%), sabinene (19.3%) and low in camphor (5.8%) characteristic of 'Cal A'. The Yucca Valley population (Cal B) was extremely diverse in its oils. It does have moderate amounts of  $\alpha$ -pinene and sabinene and a high concentration of camphor (21.9%), but with one individual (YV-3, Adams 12207) that is practically devoid of mono-terpene hydrocarbons (Table 2). The latter oil composition is the most unusual I have ever encountered in

*Juniperus*; it appears that mono-terpene synthase(s) have been inactivated in this individual. The good population would be worthy of additional study, especially as regards to examine terpene synthases and their expression. The oils of the two Arizona populations were very similar, so only the southeastern-most population (AZ, Table 2) is shown in detail. The Arizona oil is very high in  $\alpha$ -pinene (45.4%), very low in sabinene(0.4%) with moderate amounts of camphor (14.7%).

To examine variation among populations in their total oil components, PCO was performed using 40 terpenes. This resulted in eigenroots that accounted for 36, 13 and 8% of the variance among populations. Ordination (Fig. 2) reveals two major groups: Central Valley, and NW Arizona - s. California desert populations. The latter group can be further subdivided into the NW Arizona and s. California desert populations (Fig. 2).

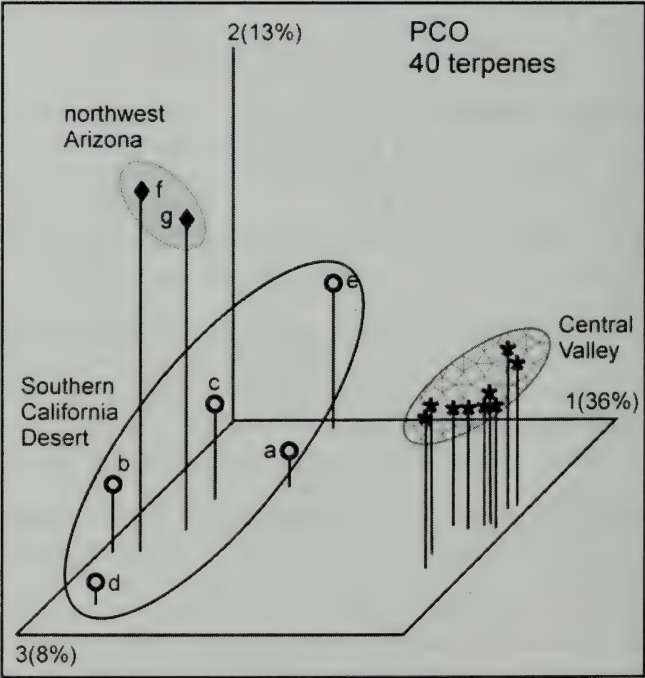


Figure 2. PCO of 16 *J. californica* populations based on 40 terpenes.



The differentiation of the Central Valley populations is clearly seen by contour mapping the clustering (Fig. 3). Notice a small difference between the more inland (1, 2, 3) and coastal range populations (6, 7, 8). The Yucca Valley population (d) is somewhat differentiated from the other southern California desert populations (a, b, c, e, Fig. 3). The NW Arizona populations form a low level group.

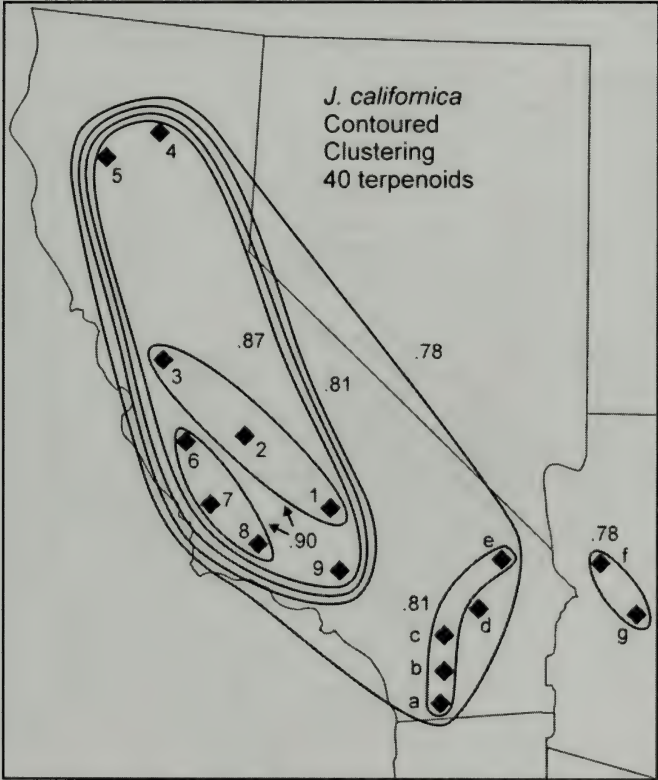


Figure 3. Contoured clustering of *J. californica* populations.

Additional insight is obtained by examining a minimum spanning network of the populations (Figure 4). An interesting aspect is that the northern populations (4, Red Bluff; 5, Lakeport) are more similar to southern Central Valley populations than to each other (note

dotted link = 0.816, Fig. 4). The Lakeport population (5, Fig. 4) has a very high secondary similarity (0.890) to the Del Puerto Canyon population (3, Fig. 4). The Central Valley group links with the Ocotillo population (a, Fig. 4) at a lower similarity (0.773). The NW Arizona populations link with Pinyon Flats CG at 0.760. One is impressed with the north-south linkages of populations in the Central Valley. In general, the sites appear more mesic as one goes northward in the Central Valley. The Red Bluff population is in a mesic oak woodland on grassy, lava rock, as is the Lakeport, which appears to be the most mesic *J. californica* population sampled.

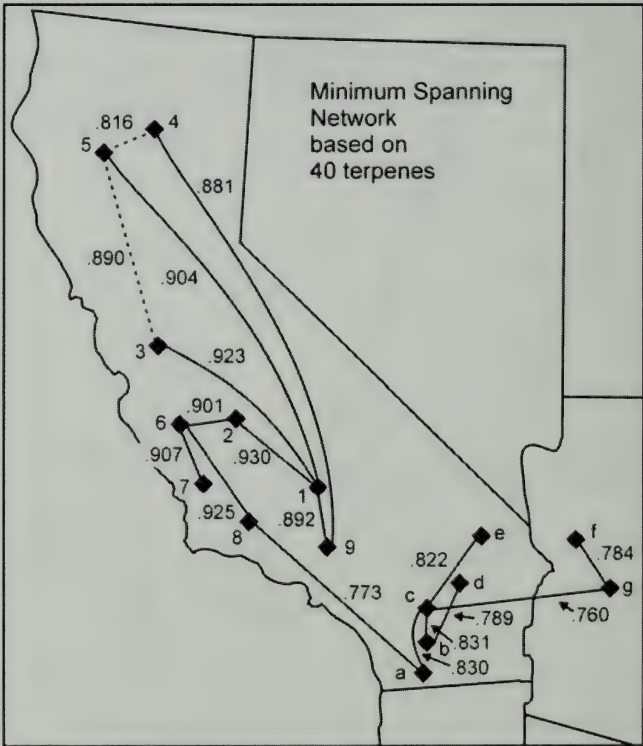


Figure 4. Minimum spanning network based on 40 terpenes. The dotted lines show secondary similarities.

Patterns of variation among individuals in the southern California and NW Arizona populations were examined by PCO using 40 terpenes. Figure 5 shows little clustering by population. The Palmdale population (9 of other figures) is part of the Central Valley group (Figs. 2-4), but very variable, so it was included. There is no evidence of intermixing of southern California desert plants with the Palmdale (9) population. However, given the diversity found among

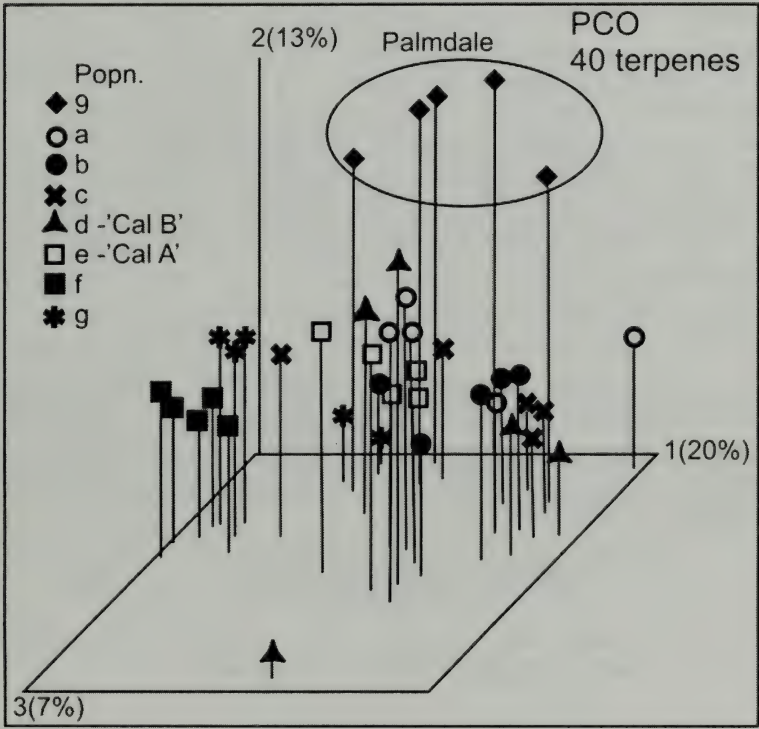


Figure 5. PCO of 40 *J. californica* individuals from the southern California desert based on 40 terpenes.

the southern California desert individuals, it might be difficult to clearly ascertain this. There is some clustering of the NW AZ individuals (particularly the Yucca, AZ plants, popn. f, Fig. 5). However, 'Cal A' (Kelso, e, Fig. 5) plants and 'Cal B' plants (Yucca Valley, d, Fig. 5) are

somewhat scattered to the center and left on PCO coordinate 1 (Fig. 5). Note the lone individual in the foreground (Fig. 5). This the unusual plant (*Adams 12207*) from Yucca Valley with essentially no monoterpenes.

It seems very unusual that the oils from plants in the northern portion of the range (Central Valley) of *J. californica* are so uniform and the oils from the southern California desert are so variable. This is suggestive of disruptive gene combinations that one sees in hybrid swarms (see Figs. 11-16, Adams, 1983). There are three other closely related junipers that occur in the vicinity of *J. californica*: *J. grandis* (San Bernardino Mtns.), *J. monosperma* (northwestern Arizona) and *J. osteosperma*, at higher elevations on mountainsides above *J. californica*. Collections and analyses of the leaf oils from these three species revealed no evidences of hybridization with *J. californica*. Additional research is needed to understand these unusual patterns of differentiation.

## CONCLUSIONS

The chemical races of Vasek and Scora (1967) were found to from a mosaic that did not fit any geographic pattern in southern California. The differentiation of the Central Valley populations appears to be due to a post-Pleistocene migration from germplasm in the southern California deserts.

## ACKNOWLEDGEMENTS

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Table 2. Comparison of leaf essential oils of *J. californica* from the Bodfish (Bod), Red Bluff (RBf), Lakeport (Lkp), Kelso (Kel), Yucca Valley (YV), Yucca Valley, - tree 3 (*Adams 12207*), and nw Arizona (AZ). Kelso and Yucca Valley are called 'Cal A' and 'Cal B' in Vasek and Scora (1967) and Adams et al. (1983). Compounds in bold face appear to separate the taxa. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. KI is the Kovat's Index using a linear approximation on DB-5 column. \* = cpds used for PCO.

KI	Component	Bod	RBf	Lkp	Kel	YV	YV-3	AZ
921	tricyclene	0.2	0.1	0.1	0.1	0.1	-	t
924	$\alpha$ -thujene*	0.5	0.3	0.3	0.9	0.5	-	0.3
932	<b><math>\alpha</math>-pinene*</b>	1.7	0.6	0.5	30.3	12.2	0.2	45.4
945	$\alpha$ -fenchene*	-	-	-	-	-	-	t
946	camphene*	0.3	0.2	0.2	0.4	0.3	-	0.5
961	verbenene*	-	-	-	-	-	-	-
969	<b>sabinene*</b>	11.7	8.5	10.0	19.3	9.0	t	0.4
974	$\beta$ -pinene*	0.2	0.1	0.1	0.8	0.4	-	0.4
988	myrcene*	1.3	1.0	1.2	2.1	1.1	1.1	1.5
1001	<b><math>\delta</math>-2-carene*</b>	-	t	0.8	0.6	-	-	0.3
1002	$\alpha$ -phellandrene*	0.2	0.2	0.2	0.2	0.2	t	t
1008	<b><math>\delta</math>-3-carene*</b>	-	-	-	-	1.3	-	t
1014	<b><math>\alpha</math>-terpinene*</b>	1.8	1.6	1.6	1.4	1.4	-	0.1
1020	p-cymene*	0.1	0.1	0.2	0.5	0.5	-	0.1
1024	limonene*	2.3	1.8	2.0	1.9	0.9	0.1	1.9

KI	Component	Bod	RBF	Lkp	Kel	YV	YV-3	AZ
1025	$\beta$ -phellandrene*	1.4	1.3	1.3	1.9	0.9	0.1	1.3
1044	(E)- $\beta$ -ocimene	0.2	0.2	0.3	0.2	0.2	-	0.3
<b>1054</b>	<b><math>\gamma</math>-terpinene*</b>	<b>2.9</b>	<b>2.7</b>	<b>2.7</b>	<b>2.4</b>	<b>2.4</b>	<b>t</b>	<b>0.2</b>
<b>1065</b>	<b>cis-sabinene hydrate*</b>	<b>0.7</b>	<b>0.7</b>	<b>0.9</b>	<b>1.0</b>	<b>1.0</b>	<b>0.3</b>	<b>0.2</b>
1067	cis-linalool oxide (furanoid)	-	t	t	t	t	-	t
1086	terpinolene*	1.0	0.9	1.0	1.1	0.9	t	0.4
1098	trans-sabinene hydrate*	0.7	0.9	0.9	1.4	1.3	-	0.3
1100	n-nonanal	-	0.3	t	-	-	-	-
<b>1112</b>	<b>trans-thujone*</b>	<b>t</b>	<b>t</b>	-	<b>0.6</b>	<b>0.5</b>	-	<b>t</b>
<b>1118</b>	<b>cis-p-menth-2-en-1-ol*</b>	<b>0.7</b>	<b>0.5</b>	<b>0.8</b>	-	-	<b>0.6</b>	<b>0.5</b>
1136	trans-p-menth-2-en-1-ol*	-	-	-	-	-	0.9	-
<b>1141</b>	<b>camphor*</b>	<b>30.3</b>	<b>43.6</b>	<b>27.8</b>	<b>5.8</b>	<b>21.9</b>	<b>1.5</b>	<b>14.7</b>
1145	camphene hydrate*	0.9	1.9	0.9	0.3	0.9	0.6	0.6
1148	citronellal*	0.5	0.2	0.5	2.3	0.6	-	1.0
1165	borneol*	t	0.1	0.2	0.1	0.4	1.4	0.8
<b>1174</b>	<b>terpinen-4-ol*</b>	<b>7.8</b>	<b>7.2</b>	<b>8.2</b>	<b>5.5</b>	<b>8.0</b>	<b>9.2</b>	<b>0.9</b>
1179	p-cymen-8-ol	t	-	-	-	-	t	-
1186	$\alpha$ -terpineol*	0.4	0.5	0.4	0.4	0.7	1.8	0.9
1195	cis-piperitol*	0.1	0.1	0.2	0.1	0.3	0.5	0.3
1207	trans-piperitol*	0.3	0.3	0.4	0.5	0.7	1.5	0.5

KI	Component	Bod	RBf	Lkp	Kel	YV	YV-3	AZ
1223	<b>citronellol*</b>	4.8	3.7	6.3	5.9	7.4	8.8	11.1
1239	carvone	-	-	-	-	0.1	0.2	-
1249	<b>piperitone*</b>	3.1	0.4	7.0	0.3	0.1	0.1	-
1257	methyl citronellate	-	0.2	0.3	-	t	t	-
1274	pregeijerene B	-	0.2	t	t	0.3	t	0.5
1287	bornyl acetate*	0.2	0.9	0.1	1.5	0.8	0.8	1.5
1298	carvacrol	-	-	-	-	-	0.4	-
1319	unknown	-	1.0	0.5	-	-	-	t
1396	duvalene acetate	-	-	-	-	-	0.2	-
1403	<b>methyl eugenol*</b>	t	-	-	0.3	0.8	0.2	0.1
1429	cis-thujopsene	-	t	t	t	t	-	t
1452	$\alpha$ -humulene	-	-	-	t	t	-	t
1505	$\beta$ -bisabolene	-	0.1	t	t	t	0.1	t
1548	<b>elemol*</b>	2.7	2.4	2.0	2.1	8.5	29.6	3.2
1555	elemicin*	0.6	0.1	t	1.1	2.5	-	1.3
1561	<b>(E)-nerolidol*</b>	0.1	0.5	t	-	-	0.3	-
1630	$\gamma$ -eudesmol*	0.3	0.3	0.3	0.4	1.0	4.1	0.5
1649	$\beta$ -eudesmol*	0.5	0.3	0.4	0.6	2.1	8.8	1.5
1652	$\alpha$ -eudesmol*	0.4	0.3	0.4	0.6	1.8	6.3	1.1
1670	bulnesol*	0.1	0.1	0.1	0.1	0.3	1.4	0.1
1746	8- $\alpha$ -11-elemodiol*	0.4	0.5	0.4	0.3	1.3	6.3	0.5



KI	Component	Bod	RBf	Lkp	Kel	YV	YV-3	AZ
1792	8- $\alpha$ -acetoxyelemol*	0.2	0.2	0.2	0.3	1.0	4.5	0.5
1931	rosa-5,15-diene(ent-)	0.1	t	t	-	-	-	-
1948	pimaradiene	0.1	t	t	-	-	-	-
1973	diterpene, <u>204</u> , 41, 93 (272)	t	t	t	-	-	-	-
1988	manoyl oxide*	10.8	6.8	11.8	-	t	t	t
2026	geranyl linalool (E,E-)	t	t	t	-	-	-	-
2055	abietatriene*	0.3	t	t	-	-	-	-
2105	iso-abienol	4.4	3.6	4.1	-	-	-	-
2145	diterpene, <u>41</u> , 69, 255, 298	-	-	-	-	-	1.9	-
2184	sandaracopimarinal*	0.7	0.5	1.2	-	-	-	-
2282	sandaracopimarinal*	0.2	0.2	0.3	-	-	-	-
2314	trans-totarol*	0.3	0.2	0.3	-	t	0.1	-
2331	trans-ferruginol	0.1	0.1	0.1	-	-	-	-

SYSTEMATIC REASSESSMENT OF THE NORTH AMERICAN  
*PHYSALIS VISCOSA* COMPLEX (SOLANACEAE)

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ABSTRACT

The North American elements of *Physalis viscosa* are reassessed taxonomically. *Physalis cinerascens* var. *spathulifolia* (Torr.) J.R. Sullivan, a dune sand taxon of Gulf Coastal Texas and closely adjacent Mexico, is elevated to specific rank as ***Physalis spathulifolia*** (Torr.) B.L. Turner, **stat. nov.** Its closest morphological relationship appears to be with the similar Gulf Coastal dune species (*P. angustifolia* and *P. walteri*) of Louisiana, Mississippi, Alabama and Florida. *Physalis mollis* var. *variovestita* is treated as ***P. cinarescens* var. variovestita** (Waterfall) B.L. Turner, **comb. nov.**, since the latter is allopatric with var. *cinerascens* and the two grade one into the other. Distribution maps of the several taxa are provided, along with an abbreviated key to the taxa concerned. *Phytologia* 93(2): 260-269 (August 1, 2011)

RESUMEN

Se revisa la taxonomía de los elementos norteamericanos de *Physalis viscosa*. *Physalis cinerascens* var. *spatulifolia* (Torr.) J. R. Sullivan, una planta de las dunas arenosas de la costa del Golfo en Texas y Tamaulipas se eleva a nivel de especie como ***Physalis spathulifolia*** (Torr.) B. L. Turner, **stat. nov.** Morfológicamente, parece cercana a *P. angustifolia* y *P. walteri*, que se desarrollan en la costa del Golfo en dunas costeras de los estados de Louisiana, Mississippi, Alabama y Florida. *Physalis mollis* var. *variovestita* se transfiere como variedad de

*P. cinerascens*, proponiéndose la nueva combinación de *P. cinerascens* var. *variovestita* (Waterfall) B.L. Turner, **comb. nov.**, dado que es alopátrica con *P. cinerascens* var. *cinerascens* y las dos se intergradan. Se presentan mapas de distribución de los diferentes taxa, y se incluye una clave abreviada de los taxa tratados.

**KEY WORDS:** *Physalis*, *P. angustifolia*, *P. cinerascens*, *P. mollis*, *P. spathulifolia*, *P. viscosa*, *P. walteri*, Texas, dune sands

Sullivan (1985) provided a detailed systematic study of the *Physalis viscosa* complex in which five species were recognized, largely based upon biogeographical and experimental crossing data: 1. *P. viscosa* (confined to South America), 2. *P. angustifolia*, 3. *P. cinerascens* (with 2 varieties), 4. *P. mollis* (with 2 varieties) and 5. *P. walteri*. Both *P. cinerascens* and *P. mollis* occur in Texas and possess populations that occur along the Gulf Coastal region; coastal populations of the former, heretofore treated as a var. of *P. cinerascens*, are treated at specific rank; populations of the latter, heretofore treated at the specific level, or as a variety of *P. mollis*, are treated as a variety of the widespread, closely adjacent, *P. cinerascens*, with which it intergrades.

A review of the taxonomy of the group is presented below, along with justifications for the nomenclature provided. Distribution maps for all of the North American taxa of the *P. viscosa* complex are provided.

#### Key to the North American taxa of the *P. viscosa* complex:

1. Leaves glabrous or nearly so.....***P. angustifolia***
1. Leaves pubescent.....(2)
2. Under surfaces of mid-stem and upper leaves densely white-tomentose, the vestiture mostly obscuring the surface of blade.....***P. mollis***
2. Under surfaces of mid-stem and upper leaves mostly moderately to sparsely pubescent, the vestiture not usually obscuring the surface of the blades.....(3)

3. Anthers equal to or shorter than mature filaments.....**P. walteri**  
 3. Anthers 1.5 times as long as mature filaments, or longer.....(4)
4. Leaf margins to some extent undulate to dentate; corollas reflexed when fully opened; widespread, south-central USA to southern Mexico.....**P. cinerascens**  
 4. Leaf margins entire; corollas not reflexed when fully opened; coastal dune sands of Texas w Louisiana and n Mexico.....**P. spathulifolia**

**PHYSALIS ANGUSTIFOLIA** Nutt., J. Acad. Nat. Sci. Phila. 7: 113. 1834. **Fig. 1**

*Physalis viscosa* subsp. *maritima* var. *elliottii* (Kunze) Waterfall f. *glabra* Waterfall 1958

Sullivan (1985) cited a number of representative specimens, most of these shown in Fig. 1. He also noted that, "This species hybridizes with *P. walteri* in peninsular Florida, and populations can be found in this state that exhibit intermediate morphology," which seems to be the case.

**PHYSALIS CINERASCENS** (Dunal) Hitchc., Spring Fl. Manhattan 32: 1894. **Fig. 2**

*Physalis viscosa* var. *sinuadentata* Schlecht. 1846

*Physalis pensylvanica* var. *cinerascens* Dunal 1852

*Physalis curassavica* L. var. *sinuadentata* (Schlecht.) Dunal 1852

*Physalis mollis* var. *cinerascens* (Dunal) A. Gray 1875

*Physalis mollis* var. *parviflora* Rydb. 1896

*Physalis saltillensis* Fernald 1900

*Physalis viscosa* var. *cinerascens* (Dunal) Waterfall 1958

*Physalis viscosa* var. *yucatanensis* Waterfall 1967

This is a widespread, highly variable, interior species, occurring on various substrates, either calcareous or sandy. It is typified by material from northeastern, Tamaulipas, Mexico. Sullivan (1985) recognized two infraspecific taxa within the complex: var. *cinerascens* and var. *spathulifolia*. We have elevated the latter to specific rank in the present paper.



We do, however, recognize a weakly differentiated, var. *variovestita*, as follows:

/ **PHYSALIS CINERASCENS VAR. VARIOVESTITA**

(WATERFALL) B.L. Turner, **comb. nov. Fig. 3**

Based upon *Physalis variovestita* Waterfall, Rhodora 60: 137. 1958.

*Physalis mollis* var. *variovestita* (Waterfall) Sullivan 1985

Sullivan (1985) comments that *variovestita* is similar to *Physalis mollis* Nuttall in morphology and flavonoid chemistry, and the two produce fertile hybrids. However, *variovestita* is recognizable because of the combination of abundant glandular hairs that are short-dendritic and long articulated, and the dark, indistinct spots in the corolla throat.

Variety *variovestita* occurs on mostly interior deep sandy soils of southern Texas and intergrades with typical var. *cinerascens* near regions of contact (but not, in our opinion, with the more northeastern *P. mollis*). This is well attested to by annotations of Waterfall and Turner on specimens at LL-TEX. Indeed, the type of *variovestita* (from Rockport, Aransas Co, Texas) is somewhat intermediate between the two taxa [assuming typical populations of the glandular-pubescent populations are best represented in Brooks and Kenedy counties, as is our surmise]. Regardless, var. *variovestita* does not appear to grade into *P. mollis* as suggested by Sullivan's classification, although the occasional glandular hairs are found intermixed with forked hairs in many interior populations of both *P. mollis* and *P. cinerascens*, these presumably the result of interspecific hybridizations with yet other taxa, as well noted by Menzel (1960). Many such specimens were annotated by Waterfall as intergrades between *P. cinerascens* and *P. variovestita*.

**PHYSALIS MOLLIS** Nutt., Trans. Amer. Philos. Soc. 5(n.s.): 194. 1837. **Fig. 4**

*Physalis viscosa* subsp. *mollis* (Nutt.) Waterfall var. *mollis* Waterfall

This taxon is an interior species, usually confined to deep sandy soils of mostly forested areas in the regions shown in Fig. 2. It is partially sympatric with *P. cinerascens* and hybridization between the two taxa can be expected. Sullivan (1985) treated the taxon as having two infraspecific taxa: var. *variovestita* and var. *mollis*; on

biogeographical grounds, we treat the former as a variety of *P. cinerascens* in the present paper, while Waterfall treated it at the specific level.

**PHYSALIS SPATHULIFOLIA** (Torr.) B.L. Turner, **stat. nov.** **Fig. 5**  
Based upon *Physalis lanceolata* var. *spathulifolia* Torr. in Emory, Rep. U. S. and Mexican Bound. Surv. 2, part 1: 153. 1859.

*Physalis viscosa* var. *spathulifolia* (Torr.) A. Gray

*Physalis cinerascens* var. *spathulifolia* (Torr.) Sullivan

This taxon has all of the earmarks of a biological species since it is confined to a consistent habitat (dune sands along the Gulf Coast) and does not appear to intergrade with its presumed closest, largely allopatric relative, *P. cinerascens*.

All of the known collections of this taxon occur along the immediate Gulf Coast in dune sands, except for a single collection from Colorado Co., Texas (Carr 19226, TEX) which was reportedly obtained on the "W side of San Bernard River floodplain, ca 0.5 mi S to SSE of mouth of Coushatta Creek, where it occurs "on deep well drained coarse sand." The plant appears to be typical of the species and is perhaps introduced there from a coastal site.

**PHYSALIS WALTERI** Nutt., J. Acad. Nat. Sci. Phila. 7: 112. 1834.

**Fig. 6.**

*Physalis elliotii* Kunze 1847

*Physalis maritima* M.A. Curtis 1849

*Physalis viscosa* var. *maritima* (M.A. Curtis) Rydb. 1896

*Physalis viscosa* subsp. *maritima* (M.A. Curtis) Waterfall var. *maritima* f. *maritima* 1958

*Physalis viscosa* subsp. *maritima* (M.A. Curtis) Waterfall f. *latifolia* Waterfall

*Physalis viscosa* subsp. *maritima* (M.A. Curtis) Waterfall var. *elliotii* (Kunze) Waterfall f. *elliotii*

According to Sullivan (1985), this species hybridizes with *P. angustifolia* in peninsular Florida, and individuals can be found in this or that population that exhibit intermediate morphology such as broadly ovate, glabrous leaves, etc.

## ACKNOWLEDGEMENTS

Distribution maps are based upon specimens on file at LL-TEX, MEXU, and specimens cited by Sullivan in her published study, these supplemented with records reported by the USDA (when deemed appropriate).

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Sullivan, J.R. 1985. Systematics of the *Physalis viscosa* Complex (Solanaceae). Syst. Bot. 10: 426-444.  
Waterfall, U.T. 1958. A taxonomic study of the genus *Physalis* in North America north of Mexico. Rhodora 60: 107-114, 128-142.

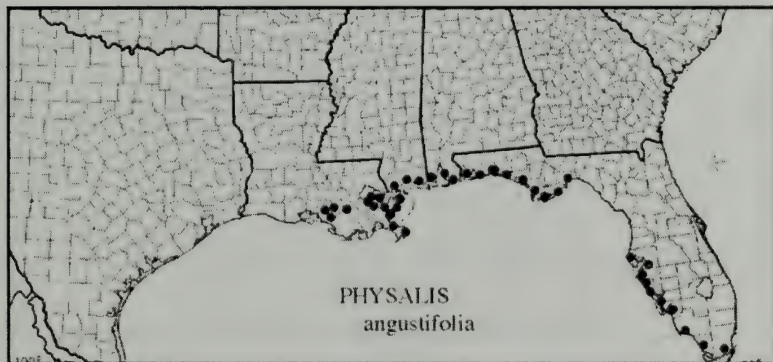


Fig. 1. Distribution of *P. angustifolia*.

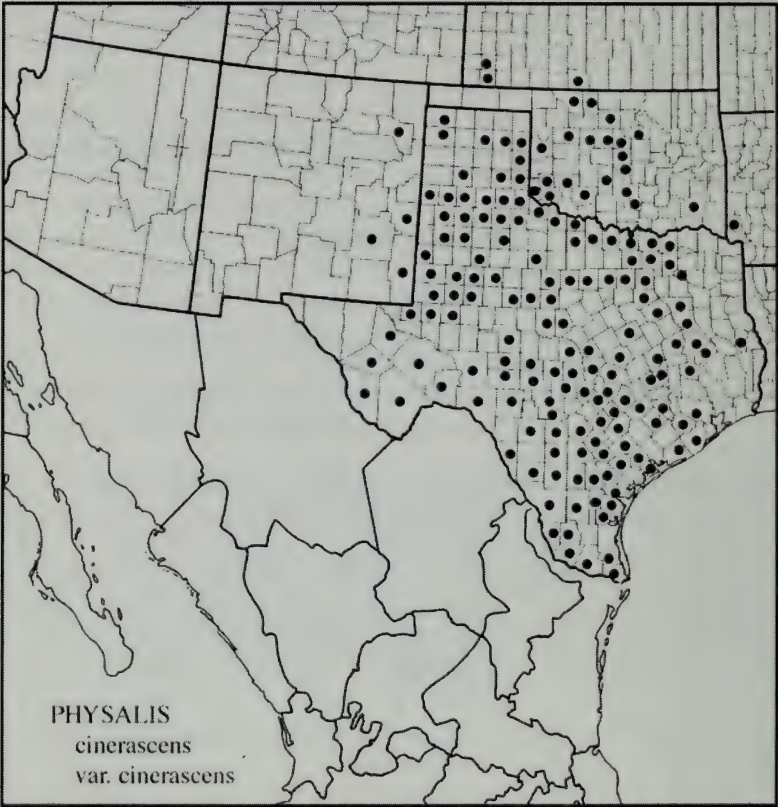


Fig. 2. Distribution of *P. cinerascens* var. *cinerascens*.



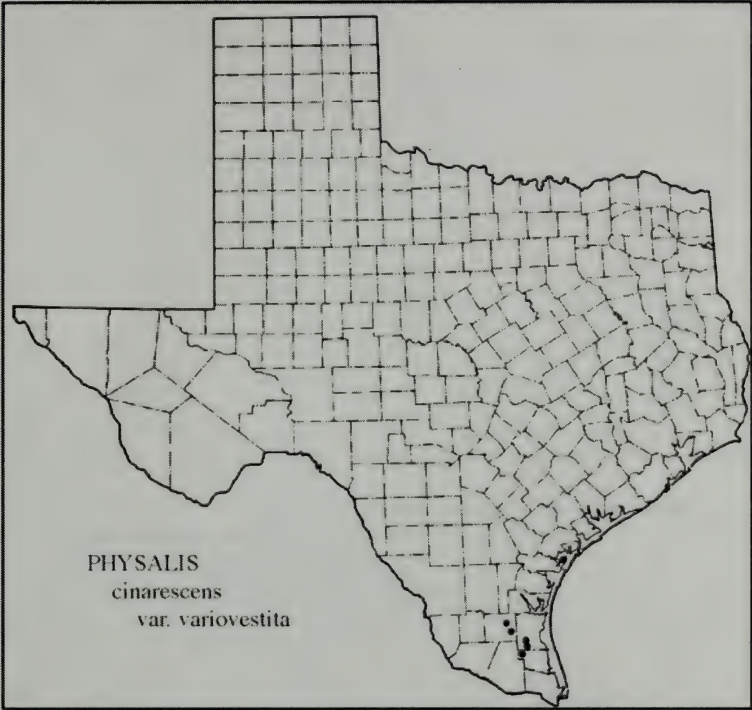


Fig. 3. Distribution of *P. cinerascens* var. *variovestita*.

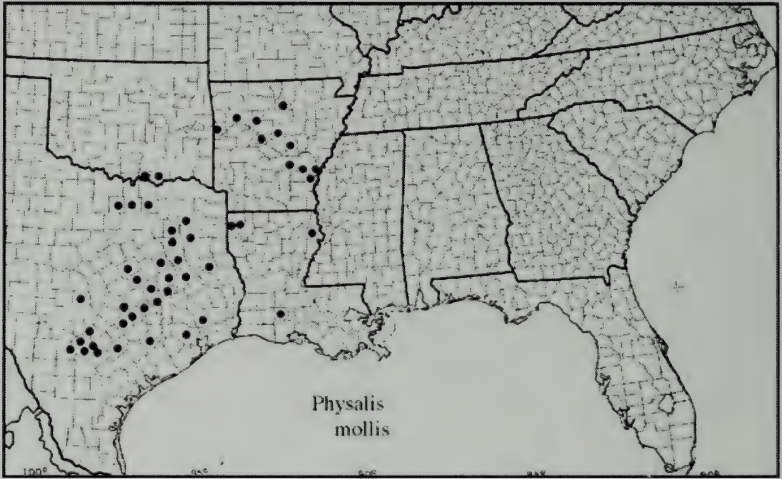


Fig. 4. Distribution of *Physalis mollis*.

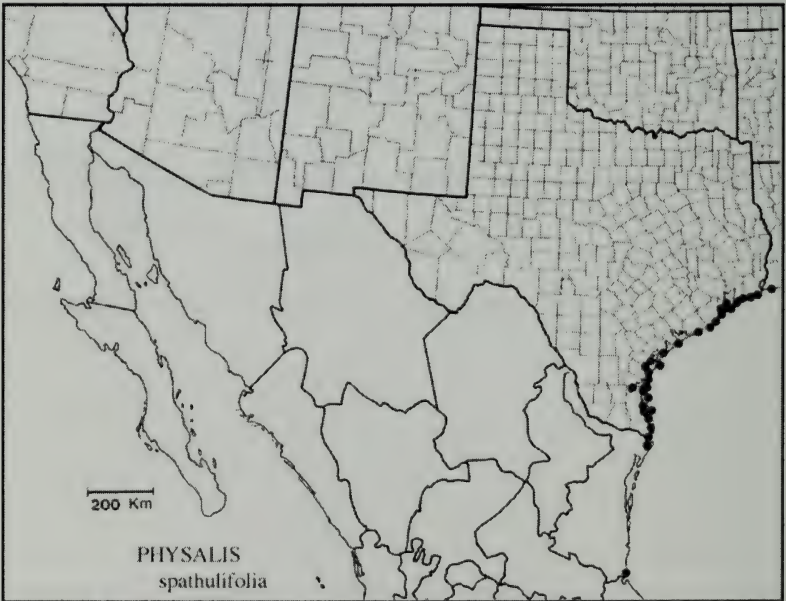


Fig. 5. Distribution of *P. spathulifolia*.

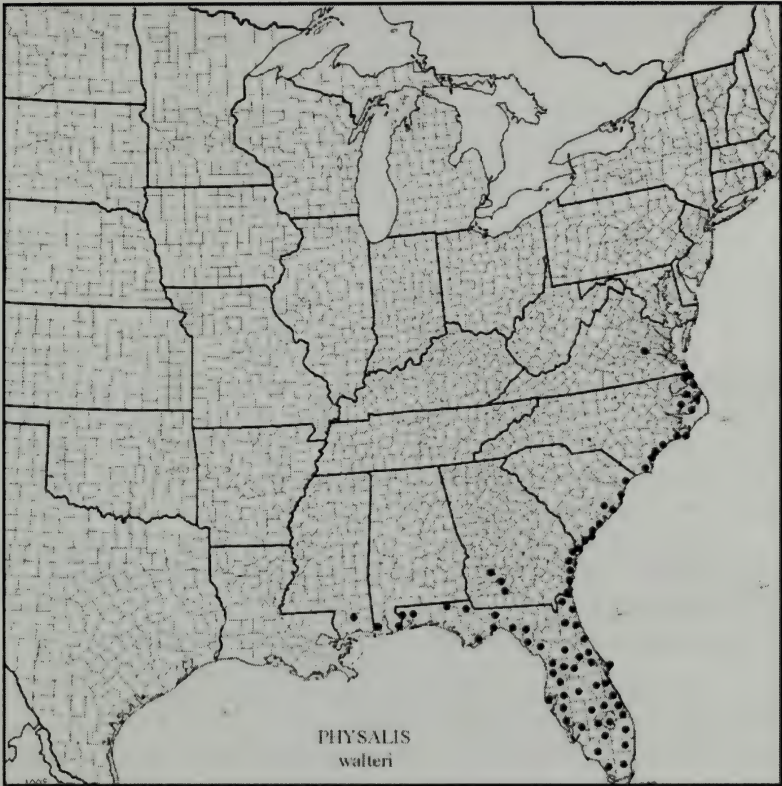


Fig. 6. Distribution of *P. walteri*.

TRANSFER OF NORTH AMERICAN *HELIANTHEMUM* TO  
*CROCANTHEMUM* (CISTACEAE): NEW COMBINATIONS

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## ABSTRACT

Transfer of *Helianthemum* (Tourn.) P. Mill. to *Crocanthemum* Spach in the Flora of North America project requires three new combinations: *Crocanthemum greenei* (B.L. Robins.) Sorrie, **comb. nov.**; *C. scoparium* (Nutt. ex Torrey & A. Gray) Millsp. var. *vulgare* (Jepson) Sorrie, **comb. nov.**; and *C. suffrutescens* (B. Schreib.) Sorrie, **comb. nov.** *Phytologia* 93(2):270-271 (August 1, 2011)

**KEY WORDS:** *Helianthemum*, *Crocanthemum*, Cistaceae, Flora of North America.

---

Recent molecular and morphological analyses of the Cistaceae (Arrington 2004) indicate that *Helianthemum* (Tourn.) P. Mill. as currently circumscribed is polyphyletic. All New World taxa are better placed within *Crocanthemum* Spach. In the forthcoming Volume 6 of Flora of North America (in prep.), I will recognize 15 species of *Crocanthemum* plus one variety. These actions require three new combinations.

1. *Crocanthemum greenei* (B.L. Robins.) Sorrie, **comb. nov.**  
Basionym: *Helianthemum greenei* B.L. Robins. Syn. Fl. N. Amer. 1(1): 191. 1895. – TYPE: U.S.A.: California, Santa Barbara County, Island of Santa Cruz, July & August 1886, Greene s.n. (holotype: ND; isotype: NY!). Note that *Crocanthemum occidentale* (Greene) Janchen (Nat. Pflanzenfam. ed 2. 21: 305), published in 1925, is based on *Helianthemum occidentale* Greene (Bull. Calif. Acad. Sci. 2: 144), published in 1886. However, Greene's epithet is a later homonym,



having been preceded by *H. occidentale* Nyman (Consp. Fl. Eur. 72), published in 1878 for a European species.

2. *Crocanthemum scoparium* (Nutt. ex Torrey & A. Gray) Millsp. var. *vulgare* (Jepson) Sorrie, **comb. nov.** Basionym: *Helianthemum scoparium* Nutt. ex Torrey & A. Gray var. *vulgare* Jepson. Man. Fl. Plants of Calif. 641. 1925. – TYPE: U.S.A.: California, Mariposa County, Coulterville, 3 July 1896, Jepson 13953 (lectotype JEPS!).

3. *Crocanthemum suffrutescens* (B. Schreib.) Sorrie, **comb. nov.** Basionym: *Helianthemum suffrutescens* B. Schreib. Madrono 5: 81. 1939. – TYPE: U.S.A.: California, Amador County, 5.5 miles southwest of Bisbee Peak, 23 May 1936, Schreiber 2243 (holotype JEPS!; isotype GH).

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Flora of North America Editorial Committee. in prep. Flora of North America North of Mexico. Vol. 6, Magnoliophyta: Cucurbitaceae to Droseraceae. Oxford University Press, New York.

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natural freeze drying - regrowth**

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Contents

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D. B. Ward. Keys to the flora of Florida - 29, <i>Spermacoce</i> (Rubiaceae) .....	275
R. P. Adams, L. E. Baker and R. N. Pandey. Seventeen years storage of juniper and spinach leaves in alcohols: Effects on DNA.....	283
R. P. Adams and T. Matraci. Taxonomy of <i>Juniperus deltoides</i> forma <i>yaltirikiana</i> in Turkey: Leaf terpenoids and SNPs from nrDNA and petN.....	293
W. H. Blackwell, P. M. Letcher, M. J. Powell and C. G. Vélez. The occurrence of <i>Blyttomyces spinulosus</i> in Alabama and Argentina, and comments on the genus <i>Blyttomyces</i> (Chytridiomycota).....	304
R. P. Adams and P. S. Shanjani. Identification of the Elburz Mountains, Iran juniper as <i>Juniperus polycarpus</i> var. <i>polycarpus</i> .....	316
B. L. Turner. <i>Brickellia enigmatica</i> (Asteraceae: Eupatorieae), A new species from north-central Mexico.....	322
M. Terry. Regeneration of <i>Lophophora williamsii</i> (Cactaceae) following mummification of its crown by natural freezing events, and some observations on multiple stem formation.....	330
B. L. Turner. Taxonomy and distribution of <i>Senecio parryi</i> (Asteraceae).....	341
B. L. Turner. A new species of <i>Decachaeta</i> (Asteraceae): Eupatorieae), from Oaxaca, Mexico.....	346
Cover Photo. <i>Lophophora williamsii</i> (peyote), natural freeze dried, and regrowth. Photo by Martin Terry.	

R. P. Adams. DNA from herbarium specimens: II. Correlation of DNA degradation with humidity.....	351
Lanner, R. M. and P. Frazier. The historical stability of Nevada's pinyon-juniper forest.....	360
S. Quintanilla-Quintero, P. Ortiz, J. E. Bernal and A. Gómez. Phylogenetic relationships among genera of the subtribe Oncidiinae (Epidendroideae: Orchidaceae) and a new genus: <i>Santanderella</i> .....	388
B. P. Hodkinson and J. C. Lendemer. The orders Ostropomycetidae (Lecanoromycetes, ascomycota): recognition of Sarrameanales and Trapeliales with a request to retain Pertusariales over Agyriales.....	407
Index to article topics and scientific names in Volume 93.....	413
Correction: Phytologia 92(2)199, 2011.....	416



**KEYS TO THE FLORA OF FLORIDA - 29,  
*SPERMACOCE* (RUBIACEAE)**

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**ABSTRACT**

*Spermacoce* (Rubiaceae) is represented in Florida by 9 species. Of these, 6 appear to be native, and 3 are introduced. One (*S. terminalis*) is endemic and is rated as threatened; none is endangered. The nomenclatural basis for certain names is detailed. One species reported for Florida is excluded. An amplified key is given to the Florida taxa. *Phytologia* 93(3): 275-282 (December 1, 2011)

**KEY WORDS:** *Spermacoce*, Rubiaceae, Florida flora.

---

Depending on how its limits are drawn, *Spermacoce* (Rubiaceae) is a genus of perhaps 150 species (Mabberley, 1997), many of the New World tropics but with representation in Africa and Asia. The species, by and large, are inconspicuous and innocuous and attract little attention. The differences between species are not striking. Keys to their identification are often too brief and artificial to be reliable. The few species that are weedy are an insignificant impediment to agricultural or horticultural objectives. None is believed to have commercial value or use. They are at times touched upon by rubiologists and of course must be treated by writers of floras. But perhaps as a result of this unimportance, the distinctions between species are little understood. The comment of R. A. Howard (Fl. Less. Antilles 6: 462. 1989) is also apt for Florida: "Few genera of the Lesser Antillean flora were represented by so many erroneous identifications or completely unidentified specimens."

The present task is to examine the species of *Spermacoce* found in Florida, to survey the scant literature, gain a confidence in the

correct use of their scientific names, and to prepare a key that carries sufficient detail to permit correct identification.

Delimitation of *Spermacoce* requires consideration of an obscure fruit character. Linnaeus (1753) and his immediate successors placed an array of related species within *Spermacoce*; all shared the 2-celled ovaries, glomerulate flowers (the source of the vernacular "Buttonweed"), and 2-seeded capsules that dehisced lengthwise into two nutlets or mericarps. Linnaeus had observed in some similar species these nutlets did not dehisce, and he held them separate as the genus *Diodia*. A later worker, Meyer (1818), went a step further. He noted the capsules of some species dehisced unevenly, with one carpel opening to expose the seed and the other remaining closed (by adhesion of the membrane that had separated the two carpels); he named these *Borreria*.

Time and further judgments have viewed these entities differently. *Diodia* remains usually treated as of generic rank, although B. Verdcourt (Fl. Trop. E. Africa. 1976) has agonized over the similarity of African *Diodia* to some members of *Spermacoce* s. str. But *Borreria* is increasingly seen as differing too slightly from *Spermacoce* to merit generic rank. [Within *Spermacoce* a lower level distinction is still maintained, as sect. *Spermacoce* and sect. *Borreria* (Meyer) Verdcourt.] As indicated by their synonymy, 5 of the Florida species were once *Borreria*, while 4 are traditional *Spermacoce*.

The best present guide to the Florida *Spermacoce* is, as is so often the case, J. K. Small's *Manual* (1933). His treatment requires adjustment in that he retained *Borreria* at generic rank, his descriptions are often not diagnostic, and two species must be added and the names of others changed. Two papers by A. B. Rendle (J. Bot. 72: 329-333. 1934; 74: 10-12. 1936), though addressing Antillean plants, forced changes in names of two Florida species (see below). An often overlooked study from Argentina by N. M. Bacigalupo (Darwiniana 17: 341-357. 1972) has excellent plates of some Florida species: *S. confusa* (her *S. tenuior*), *S. tenuior* (her *S. riparia*), and *S. glabra* (her *Spermacoceodes glabra*). Her description and plate of *S. pilifera*

Bacig. seem identical to Florida's *S. tetraquetra*, and her failure to acknowledge this earlier name suggests the two indeed may be identical. R. P. Wunderlin (Phytologia 41: 313-316. 1979) has given a brief commentary and key to 5 Florida species. D. H. Nicolson (Smithsonian Contr. Bot. 77: 197-198. 1991), reporting on Dominica, described and keyed 4 Florida species of *Spermacoce*.

Certain names used in *Spermacoce* must be changed or are under challenge. The common weed long passing under the name *Borreria laevis* (Lam.) Griseb. has undergone change of both generic name and epithet. Lamarck's type was found to be based on a specimen of *Spermacoce tenuior* (B. Verdcourt, Kew Bull. 37: 521-574. 1983), thus *laevis* is lost. Then, by merger of the genera, *Borreria* also vanishes. The name becomes *Spermacoce assurgens*.

The name *Spermacoce tenuior* L. (1753) was long used incorrectly. Linnaeus had no specimen in 1753 (a specimen presently in the Linnean Herbarium was obtained from Patrick Browne in 1758), but he gave references to three earlier authors. That of Dillenius (1732) carried a figure; by the I.C.B.N. (Art. 9.1), for early years an illustration and the specimen it was based upon may be the type. Rendle (1934) was able to locate relevant specimens in the Dillanian Herbarium at Oxford. The specimen that matched the drawing is very like the Neotropical (and Florida) *S. floridana* Urban (1913) (= *S. keyensis* Small, 1914), which name it displaces. The former *S. floridana* then became *S. confusa* Rendle (1936).

R. W. Long (Rhodora 72: 36. 1970) distinguished the Florida plant as *S. tenuior* var. *floridana* (Urban) R. Long. In the event the native Florida plant should receive recognition at specific rank, *S. floridana* Urban has priority (D. B. Ward & F. C. Craighead, Sida 14: 287-304. 1990).

Distinction of the south peninsula endemic *Spermacoce terminalis* from the pan-tropic introduced *S. verticillata* has at times been obscured (Wunderlin, 1979), but is amply confirmed by A. Herndon (Sida 12: 79-89. 1987) and J. T. Kartesz & K. N. Gandhi

(Brittonia 44: 370-371. 1992). The differences are not only of morphology but habitat selection, and suggest a long isolation by *S. terminalis* from *S. verticillata*, its probable tropical progenitor. Herndon (1987) found a curious bimodal pattern of morphology within *S. verticillata* in Florida, indicating that there may have been two introductions of this non-native plant from a variable parent population.

A question of authorship needs comment. The combination *Spermacoce densiflora* has been attributed to "Alain" or to "Liogier" by different writers. It may have been overlooked that the author was Alain H. Liogier, and that he routinely accredited new combinations with his first name. Whatever his motive, Howard (1989) cited it correctly, as "Liogier."

*Spermacoce prostrata* Aubl. (1775) has occasionally been substituted for *S. ocymoides* Burm. f. (1768). This use may first have appeared in Wunderlin (1979); it was there justified by the statement: "*Borreria* [= *Spermacoce*] *ocimoides*, however, is a totally different species confined to the Paleotropics." The name then appeared in an addendum by A. C. Clewell (1985: 523) who reported having been told (by ?) the familiar *Borreria ocymoides* -- the name he had used -- was misapplied, and was corrected to *S. prostrata*. Then Nicolson (1991) also used *S. prostrata*, with the explanation that "Fosberg and Powell (in prep.) have determined that the basionym of the usual name for this species does not apply...." Nicolson's bibliography cited these authors with an intended title and the Pacific-based journal *Allertonia* much used by Fosberg.

The basis for the use of *Spermacoce prostrata* in reference to a Florida plant is unknown. A Google search, though showing many other Fosberg publications, was unproductive. The substitute name, with the epithet *prostrata*, seems unlikely to apply to this consistently erect plant. It may well be that *S. ocymoides* is a member of a complex in which the Florida plant is not the type. But until it can be shown that differences between the type of *S. ocymoides* and the Florida plant are of specific rank, it seems imprudent to adopt a novel name for which no adequate justification is on record.



What might have been a definitive paper on *Spermacoce* and apparently was long in preparation, seems to have been lost with the 1993 death of its author. F. R. Fosberg was surely the dean of rubiacean scholars, with many papers on this family to his credit. But, following the seemingly conclusive mention by Nicolson (1991), no record of Fosberg's manuscript has surfaced.

This paper is but a weak substitute for what Ray Fosberg would have produced. It is offered in the belief that even in its imperfect state it will be useful to persons who wish to name the Florida plants but have insufficient support from published sources.

### SPERMACOCE L.      Buttonweeds<sup>1</sup>

1. Calyx with 2 long and 2 much shorter sepals (or shorter sepals lacking).
2. Terminal glomerule 1.0-2.0 cm. wide, many-flowered; corolla white; leaves with petiole 5-8 mm. long, blade broadly elliptic, 3-4 cm. long, 1.2-1.5 cm. wide, obtuse to rounded. Annual or short-lived perennial herb, to 0.5 m. Barren soils, sidewalk cracks. West and central panhandle (Escambia, Santa Rosa counties, e. to Leon Co.); rare. Spring-summer. [*Borreria densiflora* DC.]

\* *Spermacoce densiflora* (DC.) Liogier

2. Terminal (or largest) glomerule 0.5-1.2 cm. wide, relatively few-flowered; leaves ovate to linear, acute.
3. Leaves ovate-elliptic, with 3-5 pairs of lateral veins, short-petioled; stems lacking axillary clusters of small leaves; corolla shorter than calyx, white; capsules ellipsoid to obovoid. Annual herb, to 0.3 m. Nearly throughout (excl. w. panhandle); common. Summer-fall. [*Borreria ocymoides* (Burm. f.) DC.; *Spermacoce prostrata* Aubl.]

\* *Spermacoce ocymoides* Burm. f.

3. Leaves linear to linear-lanceolate, with 1-2 pairs of lateral veins, sessile or short-petioled; stems often with axillary clusters of small leaves; corolla longer than calyx, white; capsules obovoid to turbinate.

4. Corolla tube 1.2-2.5 mm. long; capsule >1.5 mm. long; leaves linear, 0.2-0.4 cm. wide; stems sprawling. Perennial herb. Seasonally wet pinelands, prairies. South peninsula (n. to Martin Co.); infrequent. All year. Endemic. Threatened (State listing). [*Borreria terminalis* Small]

**Spermacoce terminalis** (Small) Kartesz & Gandhi

4. Corolla tube 0.6-1.0 mm. long; capsule <1.5 mm. long; leaves linear to narrowly elliptic, 0.3-0.8 cm. wide; stems erect. Perennial herb, to 0.3 m. Roadsides, disturbed areas. South and central peninsula (n. to Brevard Co.); frequent. All year. [*Borreria verticillata* (L.) Meyer]

\* **Spermacoce verticillata** L.

1. Calyx with 4 subequal sepals.

5. Flowers primarily in terminal glomerule; stamens exserted; corolla white with pink throat; sepals small, withering in fruit; capsules sparsely strigose on distal half; leaves narrowly ovate. Annual or short-lived perennial herb, to 0.5 m. Low hammocks, marshes, floodplains, ditch banks. Throughout; common. Summer-fall. [*Spermacoce laevis*, misapplied; *Borreria laevis* misapplied]

BUTTONWEED.

**Spermacoce assurgens** Ruiz & Pav.

5. Flowers primarily in few-flowered axillary glomerules; stamens included; sepals persistent in fruit.
6. Corolla strongly villous in throat, white; sepals rotate in fruit, long-deltoid (length  $\pm 1.5$  times width at base); capsules glabrous. Perennial herb, to 0.6 m. Low woodlands, riverbanks. Central panhandle (Apalachicola River bottoms: Calhoun, Liberty, Gadsden counties); infrequent. Summer-fall.

**Spermacoce glabra** Michx.

6. Corolla not villous in throat; sepals erect in fruit, narrow (length >5 times width at base); capsules glabrous or bristly pubescent.
7. Leaves coarsely hirsute; stems angular, bristly hirsute on the angles; corolla white with pink tinge. Annual herb, to 0.5 m. Disturbed hammocks, pinelands, often weedy. South peninsula (Collier, Dade counties); infrequent. All year

**Spermacoce tetraquetra** A. Rich.

7. Leaves and stem scabrous or glabrous.
8. Capsules bristly pubescent, 2.0-2.5 mm. long; sepals long-deltoid (length >2 times width at base); corolla white or light pink, the lobes less than 1/2 length of tube; leaves scabrous; stems angular, scabridulous on the angles. Annual herb, to 0.5 m. Coastal hammocks. South peninsula (Monroe Co.); very rare. Summer. [*Spermacoce tenuior*, misapplied]

**Spermacoce confusa** Rendle

8. Capsules smooth to granulose, 2.5-3.0 mm. long; sepals short-deltoid (length  $\pm$  equal width at base); corolla white, the lobes longer than tube; leaves glabrous; stems scarcely angular, smooth. Annual or short-lived perennial, to 0.3 m. Rock pinelands. South peninsula (Monroe, Dade counties); infrequent. All year. [*Spermacoce floridana* Urban; *Spermacoce keyensis* Small; *Spermacoce riparia* Cham. & Schlecht.]

**Spermacoce tenuior** L.

Excluded names:

**Spermacoce tenella** HBK.

*Borreria tenella* (HBK.) Cham. & Schl.

Reported for Penascola, Escambia Co. (Small, 1933, as a comment under *Borreria terminalis*; cited by Clewell, 1985). An old record, without later confirmation.

1. This paper is a continuation of a series begun in 1977. The "amplified key" format employed here is designed to present in compact form the basic morphological framework of a conventional dichotomous key, as well as data on habitat, range, and frequency. Amplified keys are being prepared for all genera of the Florida vascular flora; the present series is restricted to genera where a new combination is required or a special situation merits extended discussion.

I am grateful to Alan Herndon, an accomplished rubiologist in his own account, for support and information.



## **SEVENTEEN YEARS STORAGE OF JUNIPER AND SPINACH LEAVES IN ALCOHOLS: EFFECTS ON DNA**

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### **ABSTRACT**

Propanol appeared better than ethanol for the long-term storage of spinach leaves, followed by hexanol and pentanol. The lowest molecular weight and yields of DNA came from spinach stored in methanol and ethanol. In an experiment with spinach leaves stored in 100, 95, 70, 50 and 25% ethanol, the 50% ethanol stored leaves appeared to yield more and higher molecular weight DNA than any other treatment. In contrast, juniper leaves stored in 100 and 95% ethanol yielded more and higher molecular weight DNA than 70, 50 or 25% ethanol. The different manner that these two very different leaves respond to storage in ethanol solutions may reflect the herbaceous nature of spinach leaves versus the woody nature of juniper leaves, as well as differences in secondary compounds. *Phytologia* 93(3): 283-292 (December 1, 2010).

**KEY WORDS:** DNA, ethanol, alcohols, 17 years preservation, degradation, juniper, spinach.

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Although silica gel is useful for the short-term preservation of leaves for subsequent DNA extraction for many plant species (see

Liston et al. (1990) apparently were the first to report on the utilization of silica gel in the field, although Doyle and Doyle (1987) earlier suggested that drying appears to be effective in preserving DNA. Silica gel is not very useful for some species such as ferns, which have very large amounts of tannins that turn the leaves yellow or brown during desiccation in silica gel (Thomson, 2002). The latter author also reported that a saturated NaCl-CTAB solution with 200 mM sodium ascorbate as an antioxidant was effective for interim preservation of DNA in Bracken fern.

One of the earliest studies on interim preservation was by Pyle and Adams (1989) in which they examined freezing, desiccation, air drying, and various liquids that are traditionally utilized to fix cell and chromosome structures (eg., Perfix preservative, paraformaldehyde, etc.). They found that none of the traditional liquids (including ethanol) preserved DNA for more than a few days. However, the same lab later found that these conclusions were invalid for ethanol (Flournoy et al., 1996). Apparently, several organic solvents result in the denaturation and precipitation of proteins, including DNases and histones. The histones are associated with DNA and when precipitated, bind to the DNA resulting in little or no DNA extracted. Flournoy et al. (1996) found that the use of proteinase digestion during grinding resulted in good DNA from short-term ethanol-preserved spinach and juniper. This information was utilized by Adams et al. (1999) in extraction of DNA from recalcitrant grasses (vetiver, wheat, maize, etc.). Field preservation of vetiver in silica gel proved effective for transport, but grinding in CTAB gave degraded DNA. However, Adams et al. (1999) found that grinding first in ethanol denatured the DNases, then CTAB extraction (with the addition of proteinase) resulted in good, genomic DNA. Fukatsu (1999) examined several organic solvents and found DNA to be well preserved in aphids (and their endosymbiotic microorganisms) in acetone, ethanol, 2-propanol, diethyl ether, and ethyl acetate for 6 months and for 2 years with acetone. King and Porter (2004) reported that ethanol was preferred for the preservation of ants for up to 6 months before extraction. Mandrioli (2008) published a useful review of DNA preservation methods in museum specimens. Some specimens of Hymenoptera yielded useful DNA after 35 years storage in 70% & 100% ethanol (at 4°C), and samples of Coleoptera had useful DNA 40 years after silica gel desiccation.

Dawson et al. (1998) reviewed several methods for the field preservation of marine invertebrate tissue and found that DMSO-NaCl (0.1 M Tris, pH 8.0, 0.02 EDTA, 0.02% [wt./vol.] CTAB in saturated NaCl to be useful; the solution was autoclaved and 20% DMSO, 0.002%  $\beta$ -mercaptoethanol and 0.25M disodium EDTA added) was the most useful and practical field method for DNA presevation (tested for up to 28 months). The reader is referred to a recent, excellent review of tissue-preservation methods (Nagy, 2010).

In 1994, we preserved leaves of *Juniperus virginiana* and spinach in various concentrations of ethanol and various mono-hydroxy alcohols ranging from methanol to decanol. After 17 years of storage at lab temperature ( $\sim 20^{\circ}\text{C}$ ), it seemed an opportune time to examine the DNA in these tissues.

## MATERIALS AND METHODS

DNA was extracted from juniper and spinach leaves (12-13 mg) by use of a Qiagen mini-plant kit as per manufacturer's instructions with the addition of 150 ug proteinase E (Sigma P6911) after the RNase incubation. Genomic DNA was visualized by agarose gel electrophoresis by mixing 3  $\mu\text{l}$  DNA extract, 3  $\mu\text{l}$  pGEM markers and 3  $\mu\text{l}$   $\lambda$ HindIII, and loading 6  $\mu\text{l}$  on a 0.6% agarose gel, then running at 100 v for 20 min. The images were captured on a Kodak Gel Logic 100 Imaging System, and profile analysis was used to determine the modal DNA size and range of DNA sizes. The DNA from some samples was subjected to PCR amplification. ITS (nrDNA) and petN-psbM amplifications were performed in 30  $\mu\text{l}$  reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15  $\mu\text{l}$  2x buffer E (petN-psbM) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200  $\mu\text{M}$  each dNTP, plus Epi-Centre enhancers with 1.5 - 3.5 mM  $\text{MgCl}_2$  according to the buffer used) 1.8  $\mu\text{M}$  each primer.

## RESULTS

The gel of spinach stored 17 years in various alcohols (Fig. 1) shows a lack of preservation in methanol and ethanol, but a surprising amount of DNA present in the propanol-stored spinach.

A comparison of the effects of different alcohols on the preservation of DNA in spinach leaves (Table 1) shows that the greatest yields were in propanol, heptanol and hexanol.

It is surprising to find the moderate yields from storage in the larger alcohols (hexanol, heptanol). The lowest yields were from methanol and ethanol. The leaf disks stored in ethanol and pentanol had lost much of their structural features and disintegrated readily upon contact with a forceps.

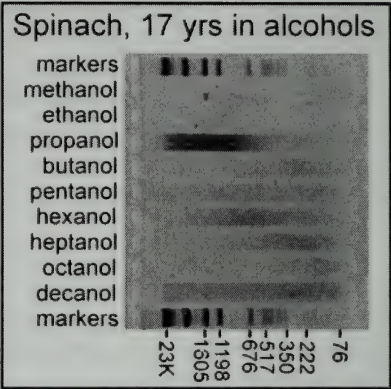


Fig. 1. Gel of spinach DNA stored 17 yrs. in various alcohols.

Table 1. Comparison of yield and DNA sizes from spinach after storage in various alcohols for 17b years.

alcohol	DNA yield (ng)	Mode(bp)	range (bp)
methanol	<0.3	1300	1650 - <76
ethanol	0.5	1000	1600 - <76
propanol	7.0	1200	~23K - 76
butanol	2.3	350	~2500 - <76
pentanol	2.3	500	~2500 - <76
hexanol	3.5	700	~2500 - <76
heptanol	3.7	250	~1500 - <76
octanol	2.3	150	~1500 - <76
decanol	3.5	300	~23K? - <76

DNA was scanned and profiles were obtained and compared with the markers (lambdaHind III + pGEM) (Fig. 2). The patterns of degradation proved very different in the various alcohols (Fig. 2).

Propanol storage clearly yielded the most and highest molecular weight DNA (Fig. 2). A second trend is seen in the increase in molecular weight from butanol and pentanol to hexanol (Fig. 2). A



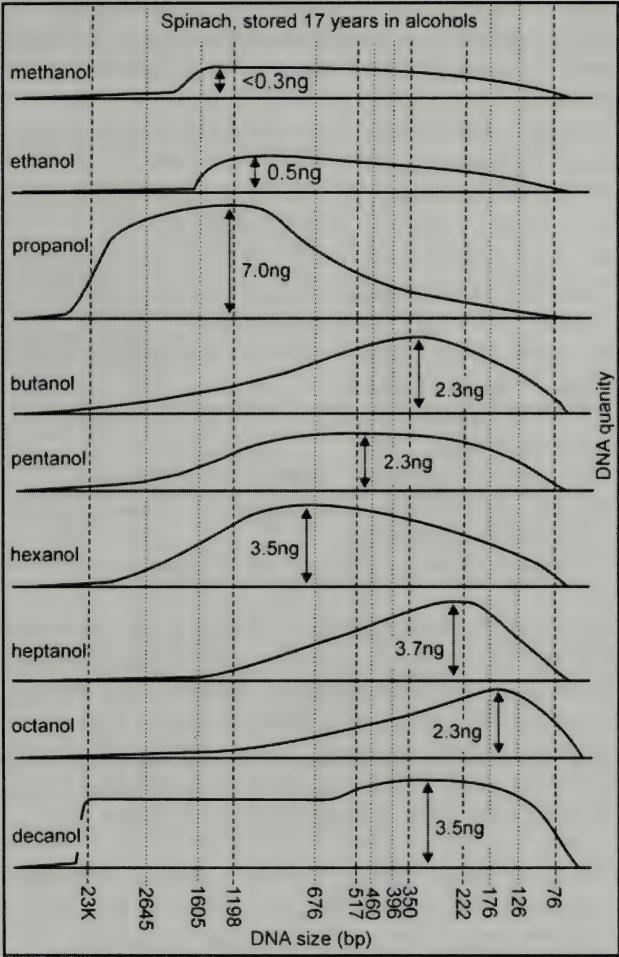


Figure 2. Profile analyses of DNA from spinach stored 17 yrs. in various alcohols.

third trend is the decrease in molecular weight from hexanol to heptanol to octanol. Finally, the last trend is the unusual curve for the decanol preserved spinach leaves. Although the mode is about 300 bp, there appears to be DNA as large as ~23K bp (Fig. 2). However, the curve is

quite flat from ~23KB to ~ 600 bp (Fig. 2), that is suggestive that some other kind of fluorescent materials may be responsible for this portion of the curve.

Figure 3 shows the almost complete loss of DNA at all concentrations except for the 50% ethanol treatment. This is surprising as this low concentration of ethanol does not seem to have been commonly utilized for preservation. Only the 100% ethanol treatment caused loss of structural integrity. It

seems likely that 100% ethanol may dissolve the lipids in the membranes, as well as precipitation of the proteins, resulting in the loss of structure.

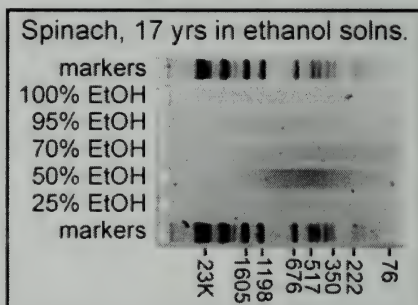


Figure 3. Gel of spinach DNA from leaves stored 17 yrs. in ethanol solns.

It should be noted that we (Flournoy et al. 1996) found large declines in high molecular weight DNA after 3 months storage in 25% and 50% ethanol. It was unexpected that the 50% ethanol storage solution was the best for spinach after 17 years. It may be, that for short-term storage (up to 3 mos.?), 100% EtOH is better but for long-term preservation of 1200-350 bp sized DNA (Flournoy et al. 1996), with 50% better for very long term storage (this study).

Flournoy et al. (1996) reported a decline in genomic DNA with the larger alcohols, with no visible DNA in a decanol solution after 3 months storage. In the current 17 year storage test, the presence of large molecules of fluorescent materials on the gel (Figs. 1, 2) may be due to plant secondary products that have cross-linked with degraded bits of DNA. Additional studies are in progress to investigate the nature of this material.

Profile analyses of spinach DNA stored in 100, 95, 70, 50 and 25% ethanol (Fig. 4) revealed that by far the most DNA was recovered from the 50% ethanol treatment. The 70% treatment is interesting in yielding a moderate amount of DNA (0.7 ng, Fig. 4) with a range from ~2500 bp to < 76 bp as well as material ranging from ~23K bp to 2500 bp. Prepping of the 'high molecular weight DNA' and PCR amplification is needed to confirm that such material yields useful DNA amplifications (in progress, RPA).

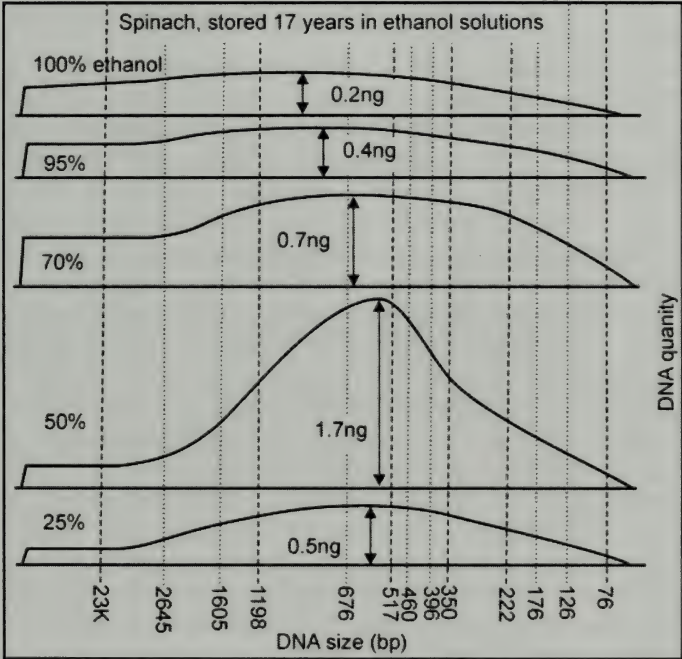


Figure 4. Profile analyses of DNA from spinach stored 17 yrs. in 100, 95, 70, 50 and 25% ethanol.

A gel showing the DNA of juniper leaves (*J. virginiana*) stored in different concentrations of ethanol (Fig. 5) reveals a very different pattern than seen with spinach (Fig. 4). Very little DNA was

obtained in 70 to 25% ethanol, with the most DNA obtained in 100% followed by 95% ethanol (Fig. 5).

No structural changes were observed in any treatment, this seems likely due to the woody nature of juniper leaves with hemicelluloses and lignans present. However, differences in chlorophyll color was quite obvious: 100% - bright green; 95% - bright green; 70% - pale green - yellow; 50% - very pale, yellow-brown; 25% - very pale (nearly clear) brown.

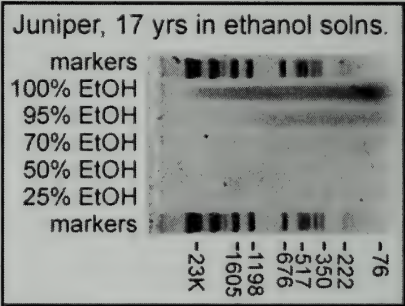


Figure 5. Gel of DNA from juniper stored 17 yrs. in 100, 95, 70, 50 and 25% ethanol.

Profile analyses (Fig. 6) of juniper leaves stored 17 yrs. in 100, 95, 70, 50 and 25% ethanol solutions had very different patterns than

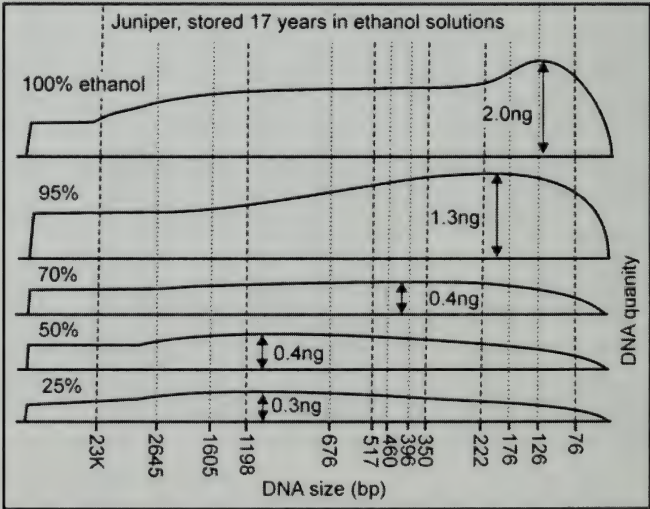


Figure 6. Profile analyses of DNA from juniper leaves stored 17 yrs. in 100, 95, 70, 50 and 25% ethanol.



seen with spinach leaves (Fig. 4). Both 100 and 95% ethanol preserved much more DNA than lower concentrations; however, there appears to be some larger DNA (2700 bp) in the 50 and 25% treatments (Fig. 6). Again, it is not known if this higher molecular weight material is useable DNA. PCR of ITS (nrDNA, ~1300 bp) was successful using DNA from the 95% treatment, but only barely successful for DNA from the 100% treatment. PCR of petN-psbM (cp DNA region, ~800 bp) was fair using DNA from 100%, and very poor for 95% treatment.

## CONCLUSIONS

Preliminary data suggest that propanol may be superior to ethanol for the long-term storage of spinach leaves, followed by hexanol and pentanol. Spinach DNA appeared to be least degraded when leaves were stored in 50% ethanol, but additional studies are needed to characterize the degraded materials. In contrast, the highest molecular weight DNA from juniper was obtained from 95 and 100% ethanol storage solutions. The different manner that these two species leaves respond to storage in ethanol solutions may reflect the herbaceous nature of spinach leaves versus the woody nature of juniper leaves, as well as differences in secondary compounds. Of course, none of the methods examined was nearly as effective in DNA preservation as desiccation followed by freezing. A interesting new paper (Akinagbe et al., 2010) found that soaking of *Picea* leaves in 70, 80, 90 or 100% ethanol before desiccating in silica gel produced nearly twice the yield of DNA as desiccation with no pretreatment. No differences were found in DNA yields after soaking in ethanol for 24, 36, or 48 h. They hypothesized that ethanol-soaking before desiccation in silica gel may have deactivated DNases, disrupted cell walls and/ or extracted certain carbohydrates from the leaves. Such effects may be a factor in how alcohols act and need further investigation.

## ACKNOWLEDGEMENTS

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**TAXONOMY OF *JUNIPERUS DELTOIDES*  
FORMA *YALTIRIKIANA* IN TURKEY:  
LEAF TERPENOIDS AND SNPS FROM nrDNA AND petN**

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**ABSTRACT**

Comparisons of SNPs of nrDNA and petN-psbM of *J. deltoides*, *J. oxycedrus*, and *J. o. f. yaltirikiana* revealed that *f. yaltirikiana* is part of *J. deltoides* (Turkey), not *J. oxycedrus* (France and Spain). Leaf terpenoids showed a similar pattern, supporting the recognition of *J. o. f. yaltirikiana* as ***J. deltoides*** R. P. Adams **f. *yaltirikiana*** (Meral Avci & Ziel.) R. P. Adams **comb. nov.** *Phytologia* 93(3): 293-303 (December 1, 2011).

**KEY WORDS:** *Juniperus deltoides*, *J. d. f. yaltirikiana*, *J. d. var. spilianus*, *J. oxycedrus*, SNPs, nrDNA, petN-psbM, terpenes, taxonomy.

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Recently, Avci and Zielinski (2008) described a new columnar form, *J. oxycedrus* L. f. *yaltirikiana* Meral Avci & Ziel. The occurrence of *J. oxycedrus* in Turkey seems problematic, as recent studies (Adams, 2004; Adams, et al., 2005) utilizing nrDNA sequencing, RAPDs, leaf terpenoids and morphology, clearly indicated that *J. oxycedrus* (*sensu stricto*) is restricted to the western Mediterranean; another, sibling species, *J. deltoides* R. P. Adams occupies the eastern Mediterranean region, including Turkey. Adams (2011) recognized both *J. deltoides* and *J. oxycedrus* in his monograph of *Juniperus*. Adams et al. (2010) analyzed the putative *J. oxycedrus*

var. *spilinanus* Yalt., Eliçin & Terzioğlu and found the taxon to be *J. deltoides* [*J. d.* var. *spilinanus* (Yalt., Eliçin & Terzioğlu) Terzioğlu]. The purpose of the present study was to compare leaf terpenoids, SNPs from nrDNA and petN-psbM and morphology of *J. o. f. yaltirikiana* with *J. oxycedrus* (France, Spain) and *J. deltoides* (Turkey) to determine if the taxon is conspecific with *J. oxycedrus* or *J. deltoides*.

## MATERIALS AND METHODS

Plant material: *J. deltoides*, Adams 9430-9432, Turkey; *J. d.* var. *spilinanus*, Adams 10264-10266, Turkey. *J. oxycedrus*, Adams 9039, 9040, France, 9053 Spain; *J. o. f. yaltirikiana*, Adams 12393-12395, Zonguldak Prov., Turkey. Voucher specimens are deposited at Baylor University (BAYLU).

*Isolation of Oils* - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

*Chemical Analyses* - Oils from each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

*Data Analysis* - Terpenoids (as percent total oil) were coded and compared among the species by the Gower metric. Principal coordinate analysis was performed by factoring the associational matrix. Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the



maximum observed value for that compound over all taxa (Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at  $-20^{\circ}\text{C}$  until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

*PCR amplification* ITS (nrDNA), petN-psbM amplifications were performed in 30  $\mu\text{l}$  reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15  $\mu\text{l}$  2x buffer E (petN-psbM) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200  $\mu\text{M}$  each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM  $\text{MgCl}_2$  according to the buffer used) 1.8  $\mu\text{M}$  each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/>). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009).

## RESULTS AND DISCUSSION

Sequencing nrDNA revealed 17 nucleotide mutational events, including two mutations that occurred in two individuals among the taxa. A minimum spanning network based on the 17 SNPs is shown in figure 1 (left). No variation was found within or among *J. deltoides*, *J. d. var. spilianus* or *J. oxycedrus* (Fig. 1, left). However, *J. oxycedrus* (France, Spain) was separated by 15 nrDNA SNPs from *J. deltoides* (Turkey) and *J. d. var. spilianus* (Fig. 1, left). The three *J. o. f. yaltirikiana* individuals were either identical to *J. deltoides* or differed by a single mutation (Fig. 1).

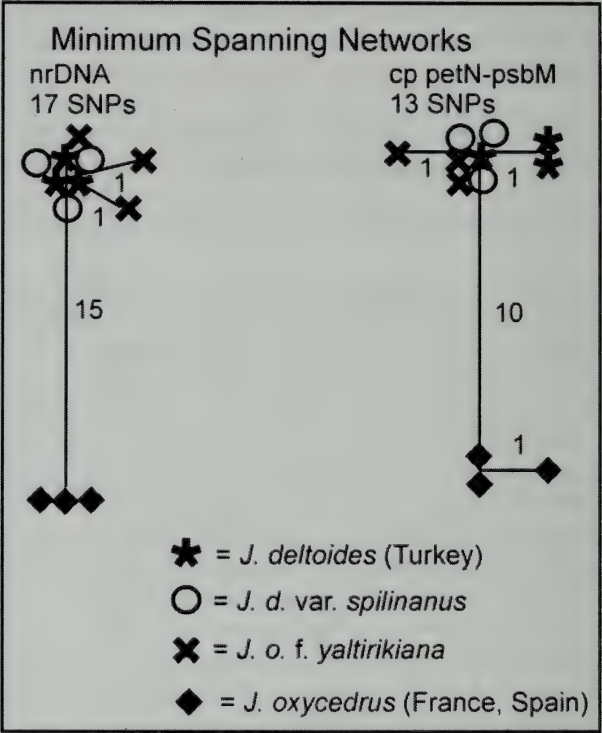


Figure 1. Minimum spanning networks based on nrDNA and petN-psbM SNPs. The numbers next to the lines are the number of SNPs.

Sequencing petN-psbM revealed 13 nucleotide mutational events that included three mutations that occurred in only single individuals among the taxa. *Juniperus oxycedrus* (France, Spain) was separated by 10 SNPs from *J. deltoides* - *J. d. var. spiliyanus* (Fig. 1, right). The three *J. o. f. yaltirikiana* individuals were either identical to *J. deltoides* or differed by a single mutation (Fig. 1). It is clear from nrDNA and petN-psbM data that *J. oxycedrus* f. *yaltirikiana* is conspecific with *J. deltoides*.

## Leaf terpenoids

The terpenoids of *J. o. f. yaltirikiana* are more like *J. deltoides* than *J. oxycedrus* (Table 1). Note the concentrations of  $\beta$ -phellandrene, trans-pinocarveol, myrtenal, carvone,  $\alpha$ -terpinyl acetate, 2-tridecanone,  $\alpha$ -muurolene,  $\alpha$ -copaen-11-ol,  $\alpha$ -calacorene, cadalene, germacrene B, dodecanoic acid, salvial-4-(14)-en-1-one, hexadecane, unknown sesquiterpene (AI1619), unknown C15-dienol acetate (AI1674), (2E,6E)-farnesol, (2E,6Z)-farnesol, 1-octadecene, nootkatone, sandaracopimara-8(14),15-diene, epi-13-manoyl oxide, sandaracopimarinal, 1-docosene and phytol acetate (Table 1). It is interesting that some *f. yaltirikiana* compounds are quantitatively more similar to *J. oxycedrus* than *J. deltoides*:  $\alpha$ -pinene, p-cymene, cis-p-menthal-2,8-dien-1-ol, caryophyllene oxide, humulene epoxide II, epi- $\alpha$ -cadinol,  $\alpha$ -cadinol, germacra-4(15),5,10(14)-triene-1-al, heptadecane, and tricosane (Table 1). The leaf oil of *f. yaltirikiana* contains several unique (to this data set) compounds: tetradecane, dodecanol,  $\beta$ -atlantol, benzophenone, muurola-4,10(14)-dien-1- $\beta$ -ol, geranyl linalool, methyl linoleate, heneicosane, and abietal (Table 1).

## Morphology

The *f. yaltirikiana* appears to differ from *J. deltoides* only by their columnar shapes. Adams (1982) found that *J. scopulorum* var. *columnaris* Fassett growing near a burning coal seam had columnar shapes. The coal seam has been burning since about 1880. Murphy and Holden (1979) propagated 25 columnar trees from the site and grew them in a smoke-free area. None of the cuttings produced columnar trees, but rather the typical, pyramidal trees of *J. scopulorum*. They concluded that ethylene from the burning coal induced the columnar shape. But, of course, other gasses from the burning coal ( $\text{SO}_2$ , NO and CO) may affect plant growth. Columnar trees of *Juniperus scopulorum* are also found downwind of coal burning plants, Butte, MT (Adams, 2011).



Figure 2. T. Mataraci with *f. yaltirikiana*.

Avci (2005) reported that hard coal is being burned to produce power in the area where the columnar junipers (f. *yaltirikiana*) occur. It seems possible that the burning coal gasses may be responsible for the columnar form. One could take cuttings and grow the clones in a smoke-free region as did Murray and Holden (1979) to determine if the columnar shape is genetic or environmentally controlled.

In view of the data presented in this study, it is apparent that *J. o. f. yaltirikiana* is not related to *J. oxycedrus* (*sensu stricto*), but to *J. deltoides*. To reflect this evolutionary relationship, *J. o. f. yaltirikiana* is recognized as:

***Juniperus deltoides*** R. P. Adams **forma** *yaltirikiana* (M. Avci & Ziel.) R. P. Adams, **comb. nov.** **Basionym:** *Juniperus oxycedrus* L. forma *yaltirikiana* M. Avci & Ziel, Phytologia Balcanica 14: 38. 2008. Type: NW Turkey, E of Zonguldak, between Göbü and Türkali villages, 100-150 m, 17 Aug 2007, M. Avci s.n. (holotype: ISTO 32573).

**Distribution:** The taxon is known from the type locality. Additional specimens were collected from the type locality by T. Mataraci, *ibid* (Adams 12393-12395)

#### Key to forms and varieties of *J. deltoides*

Base of the leaf as wide as blade, stomatal bands generally not sunken. Leaves up to 17 mm long, 2.4 wide; erect-growing, spreading or prostrate shrub or tree.

1. Erect, large shrub or small tree to 12 m,
  2. Monopodial branching; pyramidal to round crown trees, leaves 9-17x1-2.4 mm.....var. *deltoides*
  2. Fastigate branching; strict trees, leaves up to 12mm long, 1.5 mm wide.....f. *yaltirikiana*
1. Prostrate shrub or small tree to 0.5-0.6 m; leaves 6-10x1-1.5mm.....  
.....var. *spilinanus*

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Table 1. Comparisons of the per cent total oil for leaf oils components of *J. deltooides*, *J. d. var. spiliannus* and *J. d. f. yaltirikiana* compared to *J. oxycedrus*, France. Components that tend to separate the taxa are highlighted in boldface. AI = Retention Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

AI	Compound	delt	spil	yalt	oxy
802	hexanal	0.6	0.1	0.5	t
855	(E)-2-hexenal	0.9	0.3	0.7	0.4
927	tricyclene	0.1	0.2	t	0.1
930	$\alpha$ -thujene	t	0.1	t	t
<b>939</b>	<b><math>\alpha</math>-pinene</b>	<b>32.7</b>	<b>34.1</b>	<b>46.3</b>	<b>53.2</b>
953	$\alpha$ -fenchene	0.3	t	t	0.1
954	camphene	0.6	0.3	0.4	0.6
960	thuja-2,4(10)-diene	0.4	0.6	t	t
975	sabinene	0.2	1.3	0.2	0.5
<b>979</b>	<b>1-octen-3-ol</b>	-	-	-	<b>0.1</b>
979	$\beta$ -pinene	3.0	0.8	3.5	2.1
991	myrcene	3.8	0.9	3.7	2.8
<b>1002</b>	<b><math>\delta</math>-2-carene</b>	<b>0.9</b>	<b>0.3</b>	<b>0.2</b>	<b>t</b>
1003	$\alpha$ -phellandrene	1.8	1.1	t	t
<b>1011</b>	<b><math>\delta</math>-3-carene</b>	<b>3.7</b>	<b>0.1</b>	<b>t</b>	<b>5.1</b>
1017	$\alpha$ -terpinene	0.1	0.2	t	t
<b>1025</b>	<b>p-cymene</b>	<b>2.3</b>	<b>2.6</b>	<b>0.7</b>	<b>0.3</b>
1029	limonene	6.0	6.4	1.4	3.5
<b>1030</b>	<b><math>\beta</math>-phellandrene</b>	<b>11.5</b>	<b>10.5</b>	<b>6.3</b>	<b>0.8</b>
1050	(E)- $\beta$ -ocimene	-	t	t	t
1060	$\gamma$ -terpinene	0.2	0.2	0.2	0.1
1070	cis-sabinene hydrate	-	0.1	-	-
1089	terpinolene	2.0	0.8	2.0	0.7
1099	linalool	0.7	-	0.4	t
1101	n-nonanal	0.5	0.1	t	t
<b>1122</b>	<b>cis-p-menth-2-en-1-ol</b>	<b>0.3</b>	<b>0.4</b>	<b>t</b>	-
<b>1123</b>	<b>trans-p-mentha-2,8-dien-1-ol</b>	<b>t</b>	-	-	-
1126	$\alpha$ -campholenal	1.3	1.2	1.0	0.8
1126	chrysanthenone	t	t	-	-
1137	trans-pinocarveol	1.3	1.0	0.9	0.4

AI	Compound	delt	spil	yalt	oxy
<b>1138</b>	<b>cis-p-mentha-2,8-dien-1-ol</b>	<b>0.1</b>	<b>0.2</b>	-	-
1141	cis-verbenol	0.4	0.4	t	t
1145	trans-verbenol	1.8	3.0	0.5	0.6
1163	trans-pinocamphone	0.1	-	-	-
1165	pinocarvone	0.6	0.3	0.3	t
1170	p-mentha-1,5-dien-8-ol	1.1	0.6	1.1	0.5
1175	cis-pinocamphone	0.1	-	-	-
1177	terpinen-4-ol	0.6	0.4	0.4	0.3
1181	naphthalene	0.3	-	0.3	0.1
1183	p-cymen-8-ol	1.0	0.6	0.4	t
1189	$\alpha$ -terpineol	1.2	0.3	1.7	0.6
<b>1196</b>	<b>myrtenal</b>	<b>0.6</b>	<b>0.6</b>	<b>0.2</b>	<b>t</b>
1205	verbenone	0.7	0.7	0.6	0.3
<b>1217</b>	<b>trans-carveol</b>	<b>0.5</b>	<b>0.9</b>	<b>0.4</b>	<b>0.1</b>
1229	cis-carveol	t	0.2	-	-
<b>1242</b>	<b>cumin aldehyde</b>	<b>0.1</b>	<b>0.1</b>	<b>t</b>	-
<b>1243</b>	<b>carvone</b>	<b>0.3</b>	<b>0.6</b>	<b>t</b>	-
1253	piperitone	t	0.2	-	-
1257	linalyl acetate	t	-	-	0.3
1264	(2E)-decenal	0.2	0.1	-	-
1289	bornyl acetate	0.9	0.5	1.4	0.7
1298	trans-pinocarvyl acetate	0.1	-	-	-
1298	carvacrol	t	0.2	t	-
<b>1299</b>	<b>(2E,4Z)-decadienal</b>	<b>0.4</b>	-	-	-
<b>1317</b>	<b>(2E,4E)-decadienal</b>	<b>0.8</b>	-	<b>0.1</b>	<b>0.1</b>
1342	trans-carvyl acetate	t	0.1	-	-
1346	trans-piperitol acetate	-	-	-	-
<b>1349</b>	<b><math>\alpha</math>-terpinyl acetate</b>	-	-	-	<b>0.2</b>
1373	$\alpha$ -ylangene	-	t	-	-
<b>1377</b>	<b><math>\alpha</math>-copaene</b>	<b>0.2</b>	-	<b>0.3</b>	-
1381	geranyl acetate	t	-	-	-
1388	$\beta$ -bourbenene	0.2	0.2	t	0.3
<b>1400</b>	<b>tetradecane</b>	-	-	<b>0.3</b>	-
1408	longifolene	0.6	-	-	-
1419	(E)-caryophyllene	1.2	0.7	0.6	0.4
1431	cis-thujopsene	0.1	-	-	-

AI	Compound	delt	spil	yalt	oxy
1455	$\alpha$ -humulene	0.8	0.5	0.3	0.3
<b>1465</b>	<b>dodecanol</b>	-	-	<b>0.3</b>	-
1480	$\gamma$ -muurolene	t	-		0.1
1485	germacrene D	0.7	-	3.7	2.3
1486	ar-curcumene	-	0.2	-	-
1494	trans-muurola-4(14),5-diene	-	0.1		-
<b>1496</b>	<b>2-tridecanone</b>	-	-	-	<b>0.3</b>
<b>1500</b>	<b><math>\alpha</math>-muurolene</b>	<b>0.4</b>	<b>1.1</b>	<b>0.4</b>	-
1514	$\gamma$ -cadinene	0.4	0.5	0.8	0.7
<b>1514</b>	<b>cubebol</b>	-	<b>0.2</b>	-	-
1523	$\delta$ -cadinene	0.4	0.8	1.6	0.4
<b>1541</b>	<b><math>\alpha</math>-copaen-11-ol</b>	<b>0.1</b>	<b>0.2</b>	<b>0.2</b>	-
<b>1546</b>	<b><math>\alpha</math>-calacorene</b>	<b>0.5</b>	<b>0.5</b>	<b>0.3</b>	-
<b>1561</b>	<b>germacrene B</b>	-	-	-	<b>0.1</b>
<b>1566</b>	<b><math>\beta</math>-calacorene</b>	<b>0.3</b>	-	-	-
<b>1563</b>	<b>(E)-nerolidol</b>	-	<b>1.2</b>	-	-
<b>1567</b>	<b>dodecanoic acid</b>	-	-	-	<b>0.4</b>
<b>1583</b>	<b>caryophyllene oxide</b>	<b>3.2</b>	<b>3.9</b>	<b>0.2</b>	<b>0.4</b>
<b>1595</b>	<b>salvial-4(14)-en-1-one</b>	-	-	-	<b>0.4</b>
<b>1600</b>	<b>hexadecane</b>	-	-	-	<b>0.3</b>
1601	cedrol	0.1	t	-	t
1608	humulene epoxide II	1.1	1.7	0.2	0.3
<b>1608</b>	<b><math>\beta</math>-atlantol</b>	-	-	<b>0.4</b>	-
<b>1619</b>	<b>sesquiterpene alcohol, M226</b>	-	-	-	<b>0.3</b>
<b>1626</b>	<b>benzophenone</b>	-	-	<b>0.2</b>	-
1627	1-epi-cubenol	0.1	0.2	-	-
<b>1630</b>	<b>muurola-4,10(14)-dien-1-<math>\beta</math>-ol</b>	-	-	<b>0.2</b>	-
<b>1640</b>	<b>epi-<math>\alpha</math>-cadinol</b>	-	<b>0.1</b>	<b>0.6</b>	<b>0.6</b>
1651	$\beta$ -eudesmol	-	-	-	t
<b>1654</b>	<b><math>\alpha</math>-cadinol</b>	-	-	<b>0.7</b>	<b>1.6</b>
1661	cis-calamenen-10-ol	-	0.2	-	-
<b>1674</b>	<b>C15-dienol acetate, M+224</b>	-	-	-	<b>1.6</b>
<b>1677</b>	<b>cadalene</b>	<b>0.1</b>	<b>0.2</b>	<b>t</b>	-
<b>1686</b>	<b>germacra-4(15),5,10(14)-triene-1-al</b>	-	-	<b>1.1</b>	<b>1.6</b>
1700	heptadecane	-	-	0.2	0.3



AI	Compound	delt	spil	yalt	oxy
1717	(2E, 6E)-farnesol	-	-	-	0.3
1746	(2E, 6Z)-farnesol	-	-	-	0.4
1790	1-octadecene	-	-	-	t
1800	octadecane	-	-	t	t
1807	nootkatone	-	-	-	0.1
1900	nonadecane	-	-	t	0.1
1966	sandaracopimara-8(14),15-diene	-	-	-	0.1
1998	manoyl oxide	1.3	5.5	2.5	6.2
2000	eicosane	-	-	t	-
2014	palustradiene (=abieta-8,13-diene)	-	0.2	-	-
2017	epi-13-manoyl oxide	-	-	-	0.1
2023	abieta-8,12-diene	-	0.3	-	0.1
2026	geranyl linalool	-	-	0.7	-
2057	abietatriene	0.1	1.4	0.8	1.2
2088	abietadiene	-	3.4	t	1.3
2095	methyl linoleate	-	-	0.3	-
2100	heneicosane	-	-	0.8	-
2154	abieta-8(14),13(15)-diene	-	0.2	-	0.2
2185	sandaracopimarinal	-	-	-	0.2
2190	1-docosene	-	-	-	0.1
2200	docosane	-	-	0.4	0.1
2218	phytol acetate	-	-	-	0.1
2300	tricosane	-	-	0.2	0.2
2312	abieta-7,13-dien-3-one	-	0.1	-	-
2313	abietal	-	-	0.3	-

THE OCCURRENCE OF *BLYTTIOMYCES SPINULOSUS* IN  
ALABAMA AND ARGENTINA, AND COMMENTS ON THE  
GENUS *BLYTTIOMYCES* (CHYTRIDIOMYCOTA)

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ABSTRACT

Our study documents the occurrence of *Blyttomyces spinulosus* (Chytridiomycetes) in Alabama and Argentina. Argentine *B. spinulosus* specimens infected zygospores of *Spirogyra*. The find of *B. spinulosus* on zygospores of *Sirogonium* in Alabama records parasitism of a new generic host. *Blyttomyces*, though originating as a generic segregate of the operculate genus *Chytridium*, was thought to be inoperculate. However, an operculum, a structure often difficult to observe but of significance in assessing generic affinities, is here demonstrated in specimens identifiable as *B. spinulosus*. The genus *Blyttomyces* and taxonomic problems involving several species are discussed. *Phytologia* 93(3): 304-315 (December 1, 2011)

**KEY WORDS:** Apophysis, chytrid, discharge pore, operculum, parasitism, resting spore, sporangium, thallus, zoospores, zygospore.

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Since its inception (Bartsch, 1939) *Blyttomyces* has seemed distinct; yet, similarities to several genera have been noted. Generic delimitation, and the number of species included, should be reinvestigated. The most recent revisionary treatment was that of Dogma (1979), a study not all-inclusive, containing several unnamed taxa. *Blyttomyces* is now in need of taxonomic revision, but such an undertaking is premature; more collection and observation (as presented here) should be done, and more information (molecular data,

e.g., James et al., 2006) gathered. Although we do not here attempt a revision, we consider taxonomic questions concerning the genus and its species.

### THE OCCURRENCE OF *BLYTTIOMYCES SPINULOSUS*

*Blyttiomycetes spinulosus* is known from wide-spread locations: sites in Europe (including parts of Scandinavia), China, Japan, the Philippines, Cuba, and the central northern United States (Wisconsin and Michigan). We here report *B. spinulosus* from the southeastern United States. Specifically, we found this chytrid (collections WB124, WB128), March-April 2000, in central West Alabama, in standing water of a road-side ditch along Hwy 17 in northern Sumter County (approximately 1 mi. south of Geiger, AL). This is a new geographic record for *B. spinulosus*, and its occurrence on zygospores of *Sirogonium* (Zygnemataceae) documents a new algal host. This chytrid appears limited to parasitism (and possibly saprophytism, cf. Bartsch, 1939) of members of the Zygnemataceae. Previous reports noted the occurrence of *B. spinulosus* on zygospores of *Mougeotia*, *Zygnema*, *Zygnomopsis*, and particularly *Spirogyra* (cf. Sparrow, 1960; Dogma, 1979). *Sirogonium* is similar to *Spirogyra* but is distinguished by essentially parallel (not spiraled) plastid bands in the cell, and by shorter, broader conjugation connections (cf. Smith, 1950, fig. 213). We also observed one instance of infection of zygospores of *Spirogyra* by *B. spinulosus* at the Alabama locality.

Argentine specimens of *Blyttiomycetes spinulosus* are from "Pozo Verde" path, Villa General Belgrano, Córdoba Province; collection by C. Vélez, on 7-15-2008. This first, formal report of *B. spinulosus* from Argentina considerably expands the distribution record of this species. However, since *B. spinulosus* is geographically widespread, and habitats in which it may occur are common, future collecting will likely fill in gaps of an apparently disjunct distribution. Whereas Alabama collections involve a new host, *Sirogonium*, the Argentine specimens further confirm the occurrence of *B. spinulosus* on what is perhaps its main host worldwide, *Spirogyra*. The Argentine and Alabama specimens, and those recorded in the literature, are generally similar in appearance—not suggestive of distinct morphological varieties.

*Blyttimyces spinulosus* is distinctive, though not unique among chytrids, in often forming an elongate, proximal, rhizoidal ("haustorial") tube or "stalk," cf. Figs. 1 and 3, developed from the germ-tube of the encysted zoospore. This stalk-like tube connects the sporangium (usually epibiotic, on the surface of the host algal cell) with an apophysis (two or three apophyses may occur in tandem). The apophyseal system is typically intramatrical (in the host zygospore); however, an apophysis may occur external to the zygospore, i.e., between the parent algal cell wall and the contained zygospore wall. In some cases, particularly when the zygospore is more or less appressed to the parent algal wall, the "rhizoidal stalk" can be abbreviated or essentially lacking (cf. Fig. 5). Both elongate and "reduced" rhizoidal tubes were illustrated by Bartsch (1939) for *B. spinulosus*.

In *Blyttimyces*, this (sometimes elongate) proximal rhizoidal tube appears, in part, to serve to position the endobiotic system (apophysis and rhizoids) within the host zygospore. A comparable stalked structure (if somewhat more inflated or sac-like in appearance) is found in *Chytridium olla*, parasitic on oogonial contents of *Oedogonium* (cf. Denis, 1926; Sparrow, 1960, 1973; Vélez et al., 2011). Stalked, subsporangial structures may be seen in other chytrids as well. The often elongate sporangium of the saprophytic genus *Cylindrochytridium* (cf. Karling, 1977, pl. 85) may adjoin the rhizoidal system, or be subtended by an intervening cylindrical stalk. The stalk of *Cylindrochytridium*, possibly of nutritive (initially) and supportive function, can resemble the sporangium in size and shape, becoming separated from it by a transverse wall; it is thus plausible to think of this stalk as derived from a structure which is fundamentally sporangial in nature. The somewhat thickened, cup- or stalk-like base (not always evident) of the often aculeate sporangium of *Obelidium* appears to have a supportive function (cf. Karling, 1977, pl. 43, fig. 10). Such haustorial, positioning, or supporting structures (considering a number of examples) apparently have not been accorded a special name. Since it is not clear that all these structures are morphologically (or functionally) comparable, it is perhaps still ill-advised to coin such a name.

Although drawings of *Blyttimyces spinulosus* are available (Bartsch, 1939; Sparrow, 1960; Karling, 1977; Dogma, 1979), the



photographic record is sparse; hence, we present our photographs: Figs. 1-4, for Alabama specimens, and Figs. 5-6, for Argentina specimens. Note, in Argentine material, the documentation of zoospore discharge (Fig. 5). The observation of a true sporangial operculum ("lid," initially present over the discharge pore of the zoosporangium, Figs. 2, 6) is here reported in *B. spinulosus* (Blytt's, 1882, report of an operculum apparently involved a case of mistaken morphological identity, as subsequently discussed). Our find of an authentic operculum is taxonomically significant, supporting speculations (Bartsch, 1939; Dogma, 1979)—even though *Blyttimyces* was thought to be inoperculate—of similarity to the operculate genus *Chytridium*. An apophysis may be seen (Figs. 2, 4, 5), generally resembling that of *Chytridium lagenaria* (cf. Karling, 1936; Blackwell et al., 2002). The intramatrical resting spore (Figs. 3, 4) likewise suggests similarity to *Chytridium*. The apical portion of the future sporangium (developing zoospore cyst) exhibits a thickening (cf. Fig. 4) which will become, or contribute to, the apiculus (a cap-like excrescence, generally at the apex of the sporangium). The apiculus (Figs. 2, 3, 5), discharge pore (Figs. 3, 6), and spiny sporangial wall (e.g., Fig. 6) are also photographically illustrated. Sporangial spines can be distinctive or quite small (cf. Dogma, 1979). Consistent with our observations, drawings of spines of *B. spinulosus* by Bartsch (1939) show them to be distributed over the sporangial surface, possibly excepting the apiculus. The spines in some cases appear to occupy ridge-like areas on the surface (cf. Fig. 6).

### THE GENUS *BLYTTIOMYCES*: HISTORY AND TAXONOMIC COMMENTARY

*Blyttimyces* presents unresolved questions, including generic relationships and the number of recognized species. Similarities of this genus have been suggested to *Chytridium*, *Phlyctochytrium*, *Obelidium*, *Catenochytridium*, *Polyphlyctis* and *Canteria* (cf. Bartsch, 1939; Karling, 1977; Dogma, 1979). No single feature will always distinguish *Blyttimyces* from other chytrid genera; however, a combination of features typically present in *Blyttimyces* will usually serve to do so (listed as follows): a distinct apiculus; typically non-apical sporangial discharge, with one to several (variously placed) discharge pores; significant epibiotic and endobiotic (but usually not distinctly interbiotic) thallus development; one or more, often intramatrical

apophyses; in some cases a "haustorial" tube connecting the sporangium with the apophyseal system; branched rhizoids extending (further into the host substrate) from the "innermost" (or only) apophysis; and an intramatrical resting spore. Dogma (1979, p. 245) viewed *Blyttimyces* as "one of the inoperculate segregates of [the typically operculate genus] *Chytridium*;" *Blyttimyces* has in fact traditionally been considered inoperculate (Sparrow, 1960, may simply have accepted Bartsch's, 1939, interpretation of *B. spinulosus* as inoperculate). But, as noted, we observed an operculum in specimens of *B. spinulosus*. It is uncertain if any other species of *Blyttimyces* possesses an operculum. Nor, given a body of literature indicating *B. spinulosus* to be inoperculate, is it certain that all isolates of *B. spinulosus* will be found to possess an operculum. In further potential complication, it is possible, if improbable, that operculate and inoperculate taxa are masquerading under what otherwise seems to be a morphologically defined species. Future morphological and molecular work will be invaluable if any such determinations come to bear.

Bartsch (1939) based the new genus *Blyttimyces* and the type species, *B. spinulosus* (Blytt) Bartsch, on *Chytridium spinulosum* Blytt (1882), from Norway, and specimens Bartsch observed from Wisconsin—emphasizing in his description the presence of an apiculus and a subapical mode of spore discharge (involving a single discharge pore). Bartsch (1939) believed *Blyttimyces* to be inoperculate, concluding that Blytt (1882) had mistaken the apiculus for an operculum (Blytt, thus, describing the new species as a *Chytridium*); Bartsch noted that Blytt did not observe zoospore discharge. Sparrow (1952) continued work on *Blyttimyces* in the United States, recording *B. spinulosus* from northern Michigan. Sparrow (1952) also described *Blyttimyces laevis*, from a Michigan bog, a species (parasitic on *Zygnema*) with smooth sporangial walls (lacking the small spines of *B. spinulosus*) and a small, immediately subsporangial apophysis (apparently consistently placed). Sparrow noted that *B. laevis* sporangia could possess more than one subapical discharge pore. Sparrow and Barr (1955) described *Blyttimyces helicus*, also from Michigan, a taxon (on pine pollen, sometimes associated with *Sphagnum* debris) with distinct helical bands on the sporangium, and one or two basal or subbasal discharge pores. Sparrow (1960) emended *Blyttimyces* to include forms with more than one discharge pore, but did not mention

the observation of basal discharge. In discussing *Blyttimyces rhizophlyctidis* Dogma, a parasite on the chytrid *Rhizophlyctis rosea*, Dogma and Sparrow (1969) noted that (1-12) sporangial discharge pores (often on raised papillae) could occur at various positions on the sporangium. In this paper, Dogma transferred *Phlyctochytrium vaucheriae* (found on *Vaucheria*) into *Blyttimyces*. Dogma (1979) further emended *Blyttimyces*, noting that discharge pores could be subapical, subbasal, or at other locations on the sporangium—discounting the exact number and position of these pores, and the precise location of the apiculus, as necessarily generically meaningful.

Dogma (1979, p. 247), in consideration of species and specimens on a world-wide basis, “redefined” *Blyttimyces* to include these traits: “(a) posteriorly uniflagellate zoospores; (b) endo-exogenous thallus development; (c) epibiotic, inoperculate zoosporangium formed from the expanded portion of a functional spore cyst; (d) persistence of the unexpanded portion of the spore cyst in the form of a thickened appendage, the apiculus, on the zoosporangium; (e) apophysate endobiotic system; and (f) asexual formation of endobiotic resting spore by encystment of an apophysis.” Dogma’s redefinition, though useful, requires comment. While “(a)” is a true statement, this is not generically defining, since virtually all chytrids possess posteriorly uniflagellate zoospores. Concerning “(b),” whereas endogenous and exogenous development of the thallus occur in *Blyttimyces*, these do not appear to be of the alternating “endo-exogenous” type—described by Karling (1936) for *Chytridium lagenaria*—in which an apophysis may function as a prosperangium and contribute directly to sporangial generation or regeneration, including “internal proliferation of sporangia” (cf. Blackwell et al., 2002, 2006). As for “c,” as noted herein, at least one species of *Blyttimyces* can be operculate, a topic deserving of further study. Finally, in regard to “(f),” although one to several apophyses form, and at least one of these may develop into a resting spore (involving a thickening of the wall, Fig. 4), there is no actual encystment—i.e., in the sense of an encysting zoospore.

## CONSIDERATION OF SPECIES OF *BLYTTIOMYCES*

We do not here attempt to account for all possible taxa of *Blyttimyces*. Nonetheless, some comments on various species seem in



order. In a revision of *Blyttiomycetes*, Dogma (1979) described *B. verrucosus*, a new species from the Philippines. In all, eight formally named species of the genus were recognized by Dogma, and presented in his key. Four more potential taxa (given as *Blyttiomycetes* sp.—no binomials applied) were mentioned, but not individually distinguished in his key. The eight named species of *Blyttiomycetes* included by Dogma are broadly separated as follows (after Dogma, 1979):

Zoosporangial wall smooth

Zoospores usually uniguttulate (with one main lipid globule)

*B. vaucheriae*, *B. laevis*, *B. aureus*

Zoospores multiguttulate (with several lipid globules)

*B. harderi*, *B. rhizophlyctidis*

Zoosporangial wall ornamented

Zoospores uniguttulate

*B. spinulosus*

*B. helicus*

Zoospores multiguttulate

*B. verrucosus*

Johnson (1977) reported two species of *Blyttiomycetes* from southern Scandinavia, from aquatic habitats containing *Sphagnum*—both species of *Blyttiomycetes* being cultured on pine pollen bait. One of the species found by Johnson (1977) was Sparrow's (1952) *B. laevis*. Johnson (1977) placed *B. aureus* Booth (1969) in synonymy of *B. laevis*, based on what he considered to be taxonomically inconsequential differences in pigmentation, sporangial wall thickness, shape of the apiculus, and zoospore size and shape. To the contrary, Dogma (1979) saw these differences as grounds for distinguishing *B. laevis* and *B. aureus*. Unless such differences are shown to not be systematically meaningful—especially in the absence of molecular data—the merging of *B. aureus* and *B. laevis* does not seem warranted. Isolation of *B. laevis* on pine pollen supplements knowledge of its occurrence on *Zygnema* (cf. Sparrow, 1960).

The second species in Johnson's (1977) study, *Blyttiomycetes conicus*, was described as new. This species has conical sporangia with subbasal (often somewhat papillate) discharge pores, and which also often possess subbasal (rounded or papillate) wall ornamentations. The



subbasal discharge of zoospores in *B. conicus* distinguishes it from the subapical to lateral discharge in *B. spinulosus* (cf. Dogma, 1979), but not necessarily from the generally basal (if less papillate) discharge of *B. helicus* (cf. Sparrow and Barr, 1955). Nonetheless, the raised, subbasal ornamentations and unusual sporangial shape of *B. conicus* seem unique within the genus, and there are no spiral sporangial-wall bands as in *B. helicus*. Dogma (1979) apparently became aware of Johnson's (1977) *B. conicus* only in time to mention it in a note added in proof, not in time to include it in the body of his systematic treatment of *Blyttomyces*. However, Dogma (1979) did not question recognition of *B. conicus*, other than mentioning that its apiculus is not always strongly demarcated (cf. Johnson, 1977, p. 84). Karling (1977) discussed most of the recognized species of *Blyttomyces*, but did not mention *B. conicus* (doubtless due to timing of publication). Regardless of scant attention since Johnson (1977), *B. conicus* appears distinct.

In addition to species in the systematic treatment by Dogma (1979), *Blyttomyces conicus* and two other species, *B. lenis* and *B. spinosus* (supposedly different from *B. spinulosus*), are listed in *Index Fungorum*. There is, of course, potential for confusion given the similarity of spelling of "*spinosus*" vs. "*spinulosus*"—"B. spinosus" being intended by Dasgupta and John (1988) to apply to a different species than "*B. spinulosus*" (Blytt) Bartsch. Whether such names are to be treated as homonyms, even if not spelled exactly the same, is not presently clear (see ICBN, 2006; compare especially Articles 53.1 and 53.3). If these names were so interpreted, then *B. spinosus* Dasgupta & John (1988) would have to be rejected, being in this case the "later homonym." But this may prove a moot point, because examination of the description and discussion by Dasgupta and John (1988) of *B. spinosus* leads one to question its separation from *B. spinulosus*, and certain other species of *Blyttomyces*, by virtue (in *B. spinosus*) of a "constant position of the exit pore," and "its mode of zoospore discharge as a globular mass"—among other, perhaps less defining differences mentioned. Whereas Dasgupta and John (1988, p. 34) indicated in discussion a "constant position of the exit pore," they stated in formal description of *B. spinosus* (p. 32) that the exit pore may be "basal, lateral, subapical, apical"—not suggestive of constancy of position. As evident in our photograph (Fig. 5), zoospore release in *B. spinulosus* can occur in a globular cluster, not essentially different from

discharge described for *B. spinosus* by Dasgupta and John (1988, p. 34) as “a globular mass.” Were it not for the smooth sporangial walls (indicated by Dasgupta and John, 1988), *B. lenis* would also resemble *B. spinulosus*, occurring, as might be expected, on zygospores of *Spirogyra*. *Blyttimyces lenis* may represent specimens of *B. spinulosus* in which the characteristic small spines are reduced. Possible additional species are listed in Longcore (1996), two represented by names that may be *nomina nuda* (cf. Longcore’s listing).

It is perhaps obvious from above discussion that a number of taxa, or supposed taxa, of *Blyttimyces* are not particularly well known. We can remind in this regard that four unnamed taxa were discussed in Dogma (1979), the status of these requiring further investigation. *Blyttimyces* is thus in need of taxonomic attention, even as simply regards additional collection, and traditional morphological observation and description. An even more pressing issue, though, concerns the dearth of molecular information on the genus. Molecular data is presently available for *B. helicus*, this obtained from rDNA from a pollen culture of this chytrid (cf. James et al., 2006). The results of the attempted systematic placement of this chytrid using this molecular information, however, could only be described as inconclusive—*B. helicus* occurring in phylogenetic analyses on a branch by itself sister to the clade including the Rhizophlyctidales and Spizellomycetales. Not only is this species unresolved in taxonomic placement, but molecular information is not at this time available for any other *Blyttimyces* species. Given questions as to the operculate vs. non-operculate nature of species of *Blyttimyces* (as has been discussed)—and the diversity of presently included taxa—it is not entirely certain that the genus will ultimately prove to be monophyletic. Again, no definitive revision of this genus is possible without the availability of substantially more data.

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only be resolved by additional collection and molecular information. This work was supported in part by NSF Grant # DEB-0949305. CGV was partially supported by funds from OATs 70-07 and 65/08, FCEN, University of Buenos Aires.

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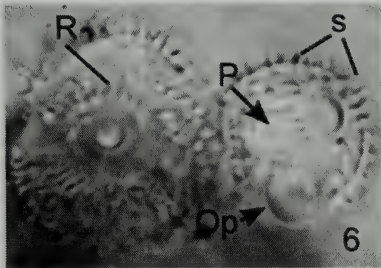
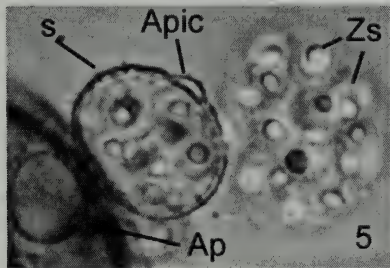
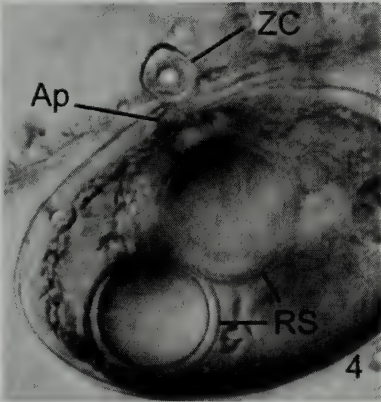
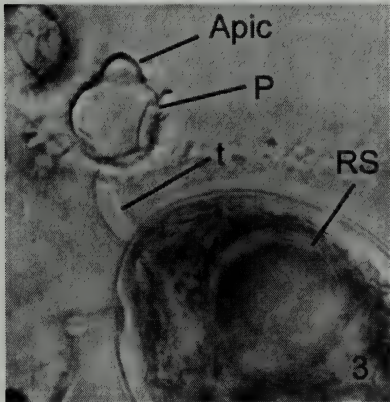
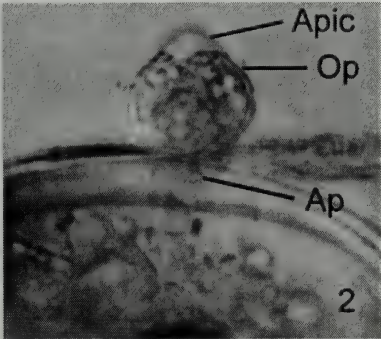
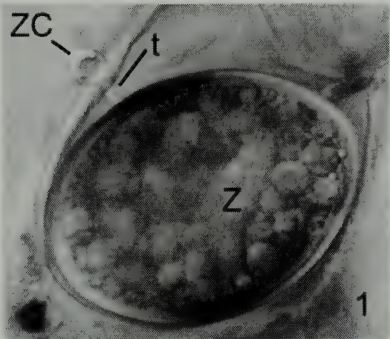


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**Figures 1-6: *Blyttomyces spinulosus*.** Fig. 1: Zoospore cyst and germination tube; zygospore of algal host (*Sirogonium*). Fig. 2: Apiculus and operculum of sporangium; apophysis visible below sporangium. Fig. 3: Apiculus and sporangial discharge pore; tube or "stalk" evident, extending into host zygospore; resting spore inside zygospore. Fig. 4: Older zoospore cyst (developing into a sporangium), apophysis below; intramatrical resting spores, the lower exhibiting defined wall. Fig. 5: Sporangial wall with small spines and apiculus; apophysis (below sporangium) in *Spirogyra* zygospore; zoospores discharged in temporarily globular cluster (to right). Fig. 6: Spiny sporangial wall (spines, in some cases, on ridge-like areas); discharge pore and operculum (previously covering pore) evident. **Abbreviations:** Ap (Apophysis), Apic (Apiculus), Op (operculum), P (discharge pore), R (ridge, bearing spines), RS (resting spore), s (small



spines on exterior of sporangial wall), t (“stalk” developed from germination tube), Z (zygospore), ZC (zoospore cyst), Zs (zoospores).



**IDENTIFICATION OF THE ELBURZ MOUNTAINS, IRAN  
JUNIPER AS *JUNIPERUS POLYCARPOS* VAR. *POLYCARPOS***

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**ABSTRACT**

The utilization of 3,714 bp from four gene regions (nrDNA, petN-psbM, trnD-trnT, trnS-trnG) was sufficient to accurately identify an unknown juniper taxon from the Elburz Mtns., Iran as *Juniperus polycarpus* var. *polycarpus*, not *J. excelsa*. The combined NJ tree (3,714 bp) showed *J. polycarpus* var. *turcomanica* to be more closely related to *J. excelsa*, than to *J. p.* var. *polycarpus*. *Phytologia* 93(2): 316-321 (December 1, 2011).

**KEY WORDS:** *Juniperus polycarpus* var. *polycarpus*, *J. p.* var. *seravschanica*, *J. p.* var. *turcomanica*, *J. excelsa*, Cupressaceae, Iran, nrDNA, petN-psbM, trnD-trnT, trnS-trnG.

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Recently, Shanjani et al. (2010) reported on the composition of the leaf essential oil from a putative *Juniperus excelsa* M.-Bieb. from the Elburz Mtns., Iran. However, the oil did not seem typical of *J. excelsa* since it was high in  $\alpha$ -pinene and missing trans-cadina-1(6),4-diene, cubebol, 1-epi-cubenol, cedrol and abietadiene. In addition, the oil contained  $\gamma$ -cadinene, elemol, and germacrene B, these not reported in *J. excelsa* by Adams (2000).

The distribution of *J. excelsa* and *J. polycarpus* is not well understood. Adams (2011) noted the occurrence of *J. excelsa* in Turkey and thence eastward into Armenia (Fig. 1.). Based on the

location of the Iranian juniper in the Elburz Mtns. (S in Fig. 1), it could be *J. p. var. turcomanica*, *J. excelsa* or *J. p. var. polycarpus*.



Figure 1. Distributions of *J. excelsa* (Greece not shown), *J. polycarpus* var. *polycarpus*, *J. p. var. seravschanica*, *J. p. var. turcomanica*. (adapted from Adams, 2011). Symbols indicate the populations sampled for each taxon. S is the site of the Iranian juniper sample in the Elburz Mtns., Iran.

The purpose of this study was to utilize DNA sequence data from nrDNA, petN-psbM, trnD-trnT, trnS-trnG regions to identify the Iranian juniper from the Elburz Mtns., Iran.

## MATERIALS AND METHODS

Plant material: *J. excelsa*, n of Eskisehir, Turkey, Adams 9433-9435, *J. polycarpus* var. *polycarpus*, Lake Sevan, Armenia, Adams 8761-8763, *J. p. var. seravschanica*, Quetta, Pakistan, Adams 8483-8485, Dzhabagly, Kazakhstan, Adams 8224-8226, Iranian juniper, Elburz Mtns., *Shanjani s. n.*, [=Adams 12603, 12604]. Voucher specimens are deposited at Baylor University (BAYLU).



**DNA Analysis** - One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at  $-20^{\circ}\text{C}$  until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA). PCR amplifications were performed in 30  $\mu\text{l}$  reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15  $\mu\text{l}$  2x buffer E (petN-psbM, trnDT, trnSG) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200  $\mu\text{M}$  each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM  $\text{MgCl}_2$  according to the buffer used) 1.8  $\mu\text{M}$  each primer. See Adams et al. (2011) for the ITS, petN-psbM, trn D-trnT and trnS-trnG primers utilized. The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/>). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

## RESULTS AND DISCUSSION

The NJ tree based on nrDNA sequences shows (Fig. 2) the Iranian juniper grouping with *J. polycarpus* var. *polycarpus* from nearby Armenia. Interestingly, *J. excelsa* and *J. p.* var. *turcomanica* show no differences in their nrDNA sequences (Fig. 2).

Analysis based on petN-psbM (cp DNA) gave a different perspective (Fig. 3). The Iranian juniper is again clearly associated with *J. p.* var. *polycarpus*, Armenia, but *J. excelsa* is in a well supported distinct clade (Fig. 3). *Juniperus p.* var. *turcomanica* is loosely associated with *J. p.* var. *polycarpus*, Armenia.



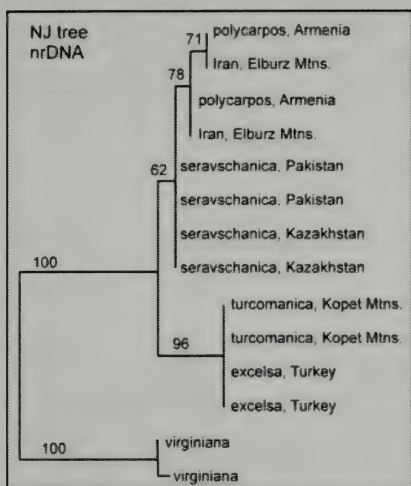


Fig. 2. NJ tree, nrDNA

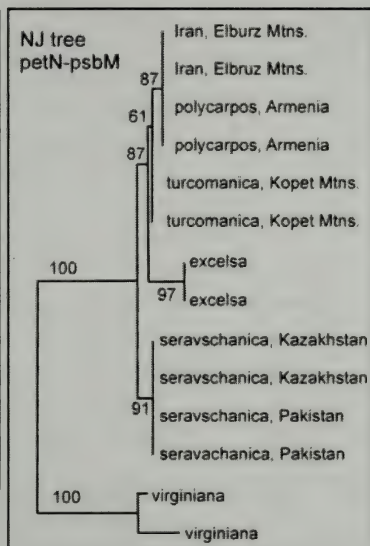


Fig. 3. NJ tree, petN-psbM.

Sequences from trnS-trnG also show the Iranian juniper in a clade with *J. p. var. polycarpus*, along with *Juniperus p. var. turcomanica* (Fig. 4). *Juniperus excelsa* is in a well-supported distinct clade.

The NJ tree based on trnD-trnT (Fig. 5) is similar to that for petN-psbM (Fig. 3) and trnS-trnG (Fig. 4) in showing the Iranian juniper in a clade with *J. p. var. polycarpus* and *J. p. var. turcomanica*. *Juniperus excelsa* is, again, in a well-supported clade (Fig. 5).

The sequences for nrDNA, petN-psbM, trnD-trnT, and trnS-trnG were concatenated to give a 3,714 bp data set. The NJ tree shows (Fig. 6) 100% support for the clade containing the Iranian juniper and *J. p. var. polycarpus*, Armenia. It is interesting to note the support for the clade containing *J. excelsa* and *J. turcomanica* (Fig. 6). Adams et al. (2008) noted the non-concordance of morphology, terpenes, RAPDs and DNA sequence data among these taxa.

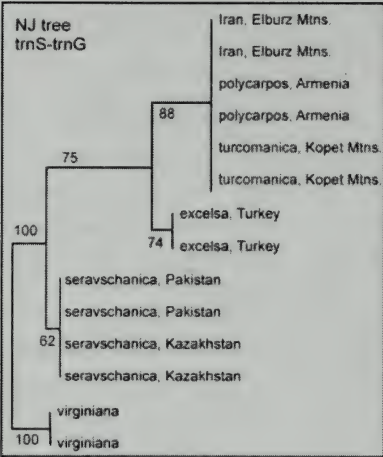


Fig. 4. NJ tree, trnS-trnG.

Figure 6. NJ tree based on 3,714 bp of sequences from nrDNA, petN-psbM, trnD-trnT, and trnS-trnG. The numbers at that branch points are bootstrap probabilities (1000 reps.).

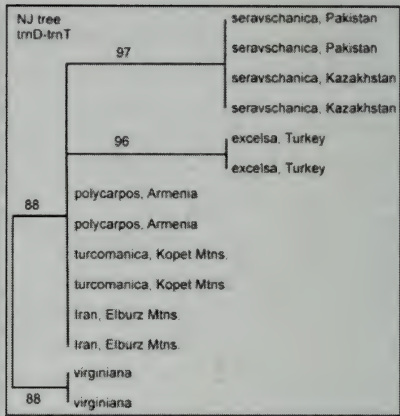
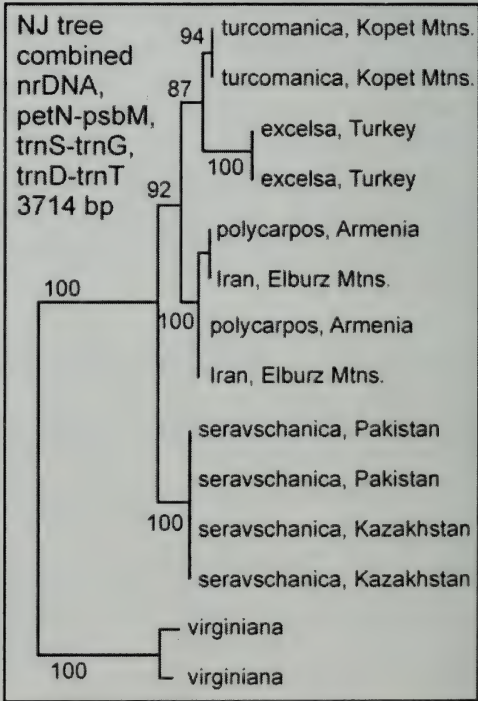


Fig. 5. NJ tree, trnD-trnT.



## CONCLUSION

The Iranian juniper from the Elburz Mtns. was found to be *J. polycarpus* var. *polycarpus* not *J. excelsa*.

## ACKNOWLEDGEMENTS

This research was supported in part with funds from Baylor University. Thanks to Tonya Yanke for lab assistance.

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**BRICKELLIA ENIGMATICA (ASTERACEAE: EUPATORIEAE),  
A NEW SPECIES FROM NORTH-CENTRAL MEXICO**

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**ABSTRACT**

A new taxon, **Brickellia enigmatica** B.L. Turner, **sp. nov.**, is described from northeastern Sonora and western Chihuahua. It is closely similar to *B. simplex* but differs in a number of characters, including smaller, more numerous heads and smaller, more densely pubescent achenes. The two taxa might also be compared with *B. odontophylla* and *B. grandiflora*, but the latter two differ in having mostly alternate leaves and more numerous heads. A key to the taxa concerned is provided, along with maps showing their distributions. A photograph of the holotype of *B. enigmatica* is also provided. *Phytologia* 93(3): 322-329 (December 1, 2011).

**KEY WORDS:** Asteraceae, *Brickellia enigmatica*, Mexico, Chihuahua, Sonora

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Routine identification of Mexican Asteraceae has occasioned the present paper, the novel species brought to my attention by recent collections assembled by Tom Van Devender and associates.

**BRICKELLIA ENIGMATICA** B.L. Turner, **sp. nov.** **Fig. 1**

*Brickelliae simplicis* A. Gray similis sed capitulis plerumque lateralibus reflexis (vs. erectis) (2-)3-5 per caulem (vs. 1-2), involucribus minoribus plerumque 10-13 mm in longitudine (vs. ca. 15 mm), et acheniis externis minoribus (2.5-3.0 mm in longitudine vs 3.5-4.0 mm) valde pubescentibus (vs. sparsim pubescentibus vel glabris).



TYPE: **MEXICO. SONORA.** Mpio. de Yecora: Rancho El Cuervo, Arroyo Escondido, near El Kipor, east of Maycoba; rocky stream canyon in pine-oak forest; 1560 m, 28° 25' 38" N, 108° 35' 58" W, 30 Sep 2003, *A.L. Reina 2003-1111* [with Van Devender, Emmitt & Eubanks] (holotype: TEX).

Perennial herbs, 30–60 cm high, from woody or somewhat tuberous rhizomes. Leaves (midstem) (2)3–9 cm long, 1–4 cm wide; petioles 1–4 cm long; blades triangular to subhastate, 3-nervate from the base, glabrous or nearly so, the lower surfaces glandular-punctate; margins irregularly dentate. Heads mostly (2)3–5, reflexed along the upper stems, the peduncles mostly 1–3 cm long; involucre bracts imbricate, the inner-most series, linear-lanceolate, 9–12 mm long. Florets white, mostly 30–35 per head; corollas glabrous, slender (ca 0.8 mm wide), 7–8 mm long; lobes 5, ca 0.5 mm long. Stamens 5, included within the corollas, extending from mid-length to near mouth. Styles swollen and pubescent at base. Achenes (outer) 2.0–2.5 mm long, markedly pubescent; pappus bristles 25–30, mostly 7–8 mm long.

Representative specimens: **MEXICO. SONORA.** 7 sheets from Mpio. de Yecora, all collected by *Reina G. & Van Devender* (96-614, 96-835, 97-1193, 98-1304, 98-1843; ARIZ, TEX); Rio Mayacoba, at crossing with new highway, 28° 23' N, 108° 46' W, 1200 m, 20 Oct 1991, *Joyal 1867* (TEX). **CHIHUAHUA.** Mpio. Madera: 11.8 mi W of Hwy 16 in Madera along the road to Rio Papigochic, 22 Sep 1984, *Sundberg 2794* (TEX); Mpio Nabogame: 1800 m, 28° 30' N, 108° 30' W, 12 Oct 1988, *Laferriere 1943* (TEX); Mpio. Ocampo: near Cascada de Basaseachic, 1950-2000 m, 17-20 Oct 1986, *Nesom & Vorobik 5606* (TEX).

Sonoran material cited above is very uniform; the Chihuahuan material is much more variable, but most key characters hold.

The species is named for its enigmatic nature, standing somewhere between *B. simplex* and *B. odontophylla*, its phyletic position uncertain.

In my treatment of *Brickellia* for Mexico (Turner 1997), this novelty will key to *B. simplex*, largely because I accepted a broad

circumscription of the species, as espoused by most workers at the time. Additionally, I had not examined typical elements of the latter, such plants not being on file at LL-TEX. Thanks to recent collections of *Brickellia* from northern Mexico by Van Devender and colleagues, I have had occasion to review the taxonomy of those taxa centering about *B. simplex*, the results presented here. Distributions of *Brickellia enigmatica* and closely related taxa are shown in Fig. 2.

Key to *Brickellia enigmatica* and closely related taxa

1. Leaves alternate throughout, or seemingly so (3)
1. Leaves opposite below, alternate above (2)
2. Involucres ca 15 mm long; heads 1 or 2, terminal, on mostly elongate, erect or semierect peduncles; outer achenes sparsely pubescent to glabrate.....**B. simplex**
2. Involucres mostly 9–12 mm long; heads 3–6, lateral on pendulous peduncles; outer achenes markedly pubescent.....**B. enigmatica**
3. Heads 5-numerous, not noticeably pendulous, arranged in terminal clusters; pappus bristles mostly 5–6 mm long .....**B. grandiflora**
3. Heads 5–10, pendulous, arranged in terminal racemes; pappus bristles (7–)8–10 mm long .....**B. odontophylla**

**BRICKELLIA GRANDIFLORA** (Hook.) Nutt., Trans. Amer. Phil. Soc., Ser. 2, 7: 287. 1841.

This widespread (Fig. 2), highly variable taxon is readily distinguished from *Brickellia enigmatica* by its terminal, numerous-headed capitulescence among yet other features. However, occasional specimens of *B. grandiflora* with few-headed capitulescences will mimic *B. enigmatica*. Much as with *B. simplex*, since the two taxa grow in close proximity, it is likely that hybrids between these occur. Scott (2006) has an excellent treatment of *Brickellia* for the Flora of North America; in this, *B. simplex* is keyed out adjacent to *B. grandiflora*. Mexican material of the latter from Nuevo León and Tamaulipas (Fig 3) might ultimately prove to represent a distinct taxon.

**BRICKELLIA ODONTOPHYLLA** A. Gray, Proc. Amer. Acad. Arts 17: 206. 1882.

As noted by Turner (1997), this taxon is “closely related to *B. simplex* [= *B. enigmatica* of present paper].” *Brickellia odontophylla* is poorly collected but appears to occur largely south of *B. grandiflora*, as shown in Fig. 2.

**BRICKELLIA SIMPLEX** A. Gray, Pl. Wright. 2: 73. 1853.

Type material of this taxon was collected by Wright in the “Hills east of Santa Cruz,” Sonora, Mexico (holotype: GH!). Most subsequent collections were obtained from the USA. Relatively few Mexican collections exist, most of these recent accessions, as follows: CHIHUAHUA. Mpio. Casas Grandes: “Ca. 2 miles E of Colonia Pacheco,” 4 Sep 1979, *Keil 13349* (ASU); 7 mi up grade from Mata Ortiz junction in oak/manzanita/grass woodland, *Spencer & Atwood 984* (TEX). SONORA. Near Observatorio Astrofísico, Sierra la Mariquita, 9.4 km (by air) NNW of Cananea, 2440 m, pine-oak forests, 19 Sep 2010, *Reina G. et al. 2010-865* (TEX).

The Sonoran collection listed in the preceding is similar to that of the holotype. The two Chihuahuan collections have 2–several-headed capitulescences approaching those of *B. enigmatica*, but their more stiffly erect peduncles and larger heads and achenes are more like those of *B. simplex*, and these are mapped as such (Fig. 3).

### ACKNOWLEDGEMENTS

I am grateful to my colleague Guy Nesom for the Latin diagnosis and for reviewing the paper. Distribution maps of Mexican taxa are based upon specimens housed at ARIZ, ASU, LL-TEX, and those reported in Robinson (1917) and the USDA web sites. Specimens of *Brickellia simplex* from Arizona and New Mexico were also examined, thanks to material on loan from ARIZ, ASU and UNM.

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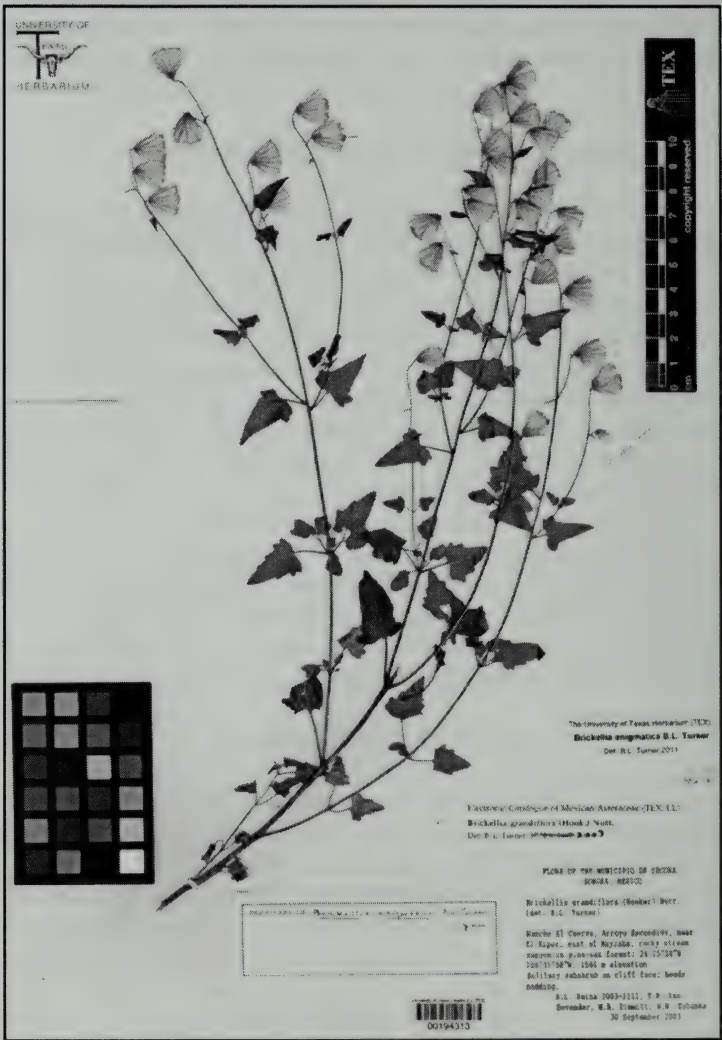


Fig. 1. *Brickellia enigmatica* (holotype).



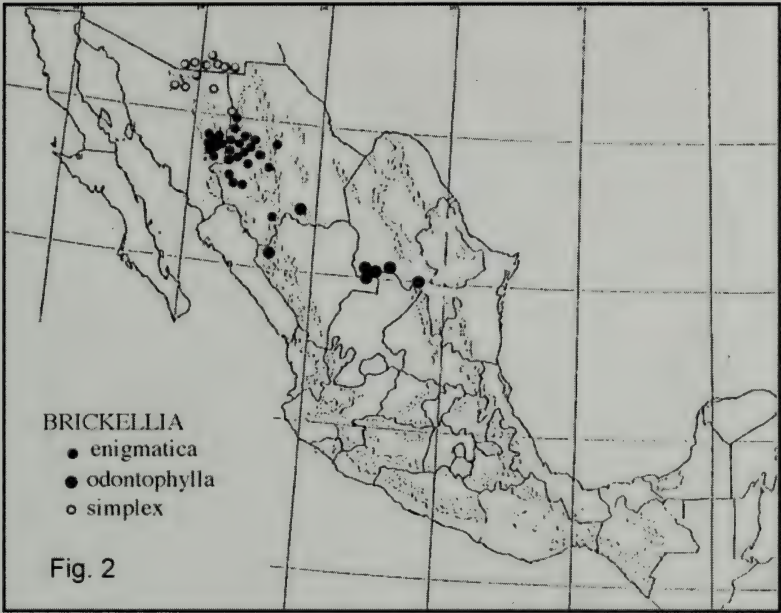


Fig. 2. Distribution of *Brickellia enigmatica*, *B. odontophylla*, and *B. simplex*.

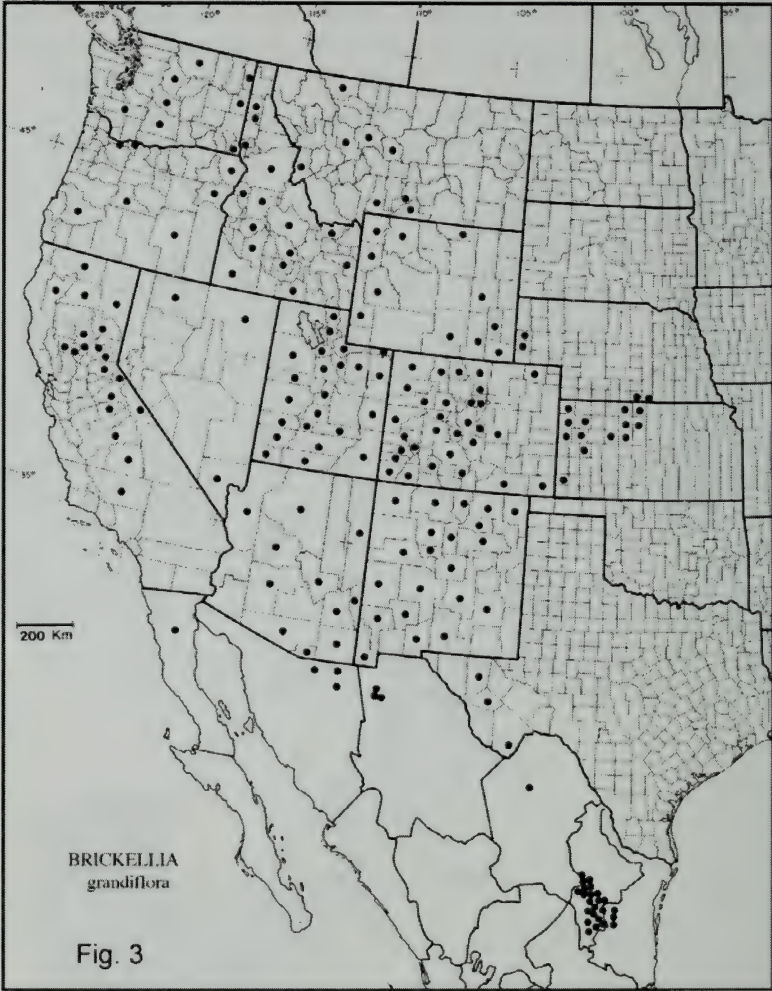


Fig. 3. Distribution of *Brickellia grandiflora*.

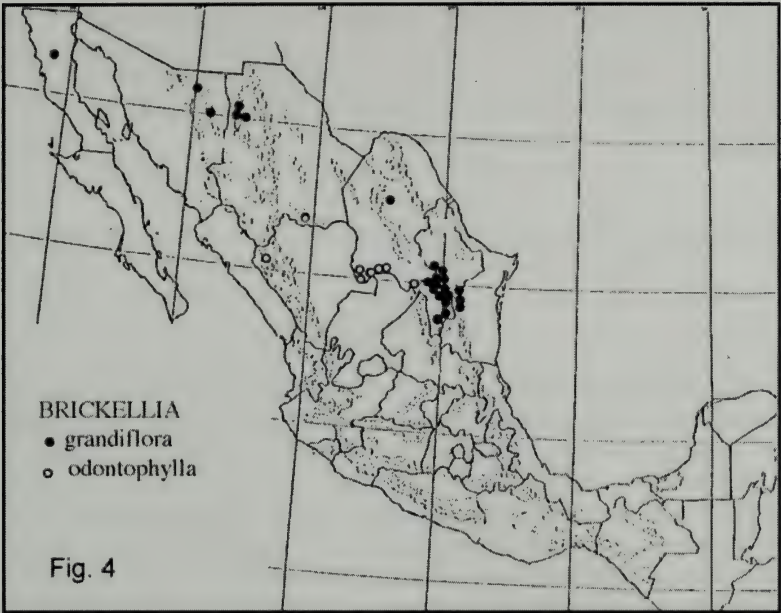


Fig. 4. Distribution of *Brickellia grandiflora* and *B. odontophylla* in Mexico.

**REGENERATION OF *LOPHOPHORA WILLIAMSII*  
(CACTACEAE) FOLLOWING MUMMIFICATION OF ITS  
CROWN BY NATURAL FREEZING EVENTS,  
AND SOME OBSERVATIONS ON MULTIPLE STEM  
FORMATION**

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**ABSTRACT**

Exposure of *Lophophora williamsii* to environmental temperatures that remained continuously below freezing, with lows estimated at or below  $-10$  to  $-15^{\circ}\text{C}$  for three consecutive days, resulted in freeze-drying of the crowns of some individuals in their natural habitat. I dug up one such plant, brought it back to the lab, planted it in a pot, watered it weekly, and monitored it for signs of life. Eight weeks later new growth was observed as incipient lateral branches from the meristems at areoles on the subterranean portion of the stem. Eleven weeks after replanting and watering, there were four such offsets on the plant. This recovery attests to the resilience of this species in the face of extreme environmental conditions. It also shows that prolonged freezing under dry conditions constitutes a natural mechanism for destruction of the apical meristem. Such meristem destruction by frost would have the same effect on lateral branching as removal of the apical meristem by human harvesting of the crown, and thus provides an alternative mechanism for the formation of pseudocespitose clusters of densely packed individuals in habitat. The concepts of cespitosity (multiple stems on a single plant) and pseudocespitosity (multiple individuals in a dense cluster) are discussed with examples. *Phytologia* 93(3): 330-340 (December 1, 2011)

**KEY WORDS:** *Lophophora*, peyote, cold tolerance, apical meristem, subterranean areoles, lateral branching, regeneration, cespitose.

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During the interval of 2–5 Feb 2011, the Big Bend region of Trans-Pecos Texas experienced a hard freeze which in many areas had



the longest continuous duration of sub-freezing temperatures in living memory (A.M. Powell, pers. comm.). This freeze occurred five months into a drought during which no biologically significant precipitation had occurred. A population of *Lophophora williamsii* (Lem. ex Salm-Dyck) J.M. Coult. (peyote) in southern Presidio County was adversely affected by the combination of drought and frost (pers. obs.).

Drought is a frequent phenomenon in Trans-Pecos Texas, and the *Lophophora* plants respond to it by contraction of the root (and possibly the underground portion of the stem, which may be substantially larger in volume than the root). This contraction pulls the crown of the plant down to, or even below, ground level, thus reducing its exposure to the evaporative forces of sun and wind (Fig. 1), as has been documented in *Ariocarpus fissuratus*, a sympatric cactus similar in habit and habitat to *L. williamsii* (Garrett et al., 2010).

The hard freeze of Feb 2011, however, was no ordinary occurrence. According to weather records for Presidio, Texas (U.S. Border Patrol, unpublished data), beginning the night of 2 Feb, the temperature remained at or below freezing continuously until the afternoon of 5 Feb, and the low temperatures in this interval were  $-9^{\circ}\text{C}$  on 3 Feb,  $-14^{\circ}$  on 4 Feb, and  $-14^{\circ}$  on 5 Feb. The altitude of Presidio is ca. 800 m. The *L. williamsii* specimens that suffered frost damage were at ca. 1300 m in the vicinity of the Chinati Mountains. That difference in altitude virtually ensures that the temperatures experienced by the population of *L. williamsii* were lower than the temperatures recorded at Presidio. Also associated with the higher altitude would be a lower atmospheric pressure, which would have increased the rate of sublimation of ice to water vapor, thus enhancing the lyophilizing effect of the cold dry air. The combination of the extreme low temperatures and the extreme duration of those low temperatures made this the most intense cold-weather event on record for this region.

## MATERIALS AND METHODS

On 24 Apr 2011, a typical eight-ribbed adult *L. williamsii* specimen measuring ca. 5 cm in diameter and growing in calcareous soil on an exposed natural terrace near the top of a slope was carefully dug up so as to cause minimal damage to the distal portion of the

taproot and to the lateral roots. The individual was selected because, as with approximately 3–5% of the plants in the population, its crown had evidently been completely freeze-dried by the prolonged hard freeze of early February. Whereas in a normal *L. williamsii* specimen in the same population the crown would consist of raised, soft parenchyma tissue covered with gray-green dermal-epidermal tissue (Fig. 1), in the case of the frost-damaged specimen the crown had been reduced to a thin layer of hard dry tissue of light reddish brown color and of the consistency of solid wood (Figs. 2a, 2b, 2c). This frost-damaged specimen retained a high density (due to high water content) in its subterranean stem and root, which suggested that the plant could be alive. Therefore it was brought back to the greenhouse, replanted in a pot, watered weekly, and monitored for signs of life.

## RESULTS AND DISCUSSION

On 16 Jun 2011, ca. 8 weeks after the specimen was replanted in the greenhouse, three offsets were visible above ground level, and by 7 Jul 2011 (ca. 11 weeks after replanting) a fourth offset had appeared above ground level (Fig. 3). Offsets are lateral branches produced by areolar meristems on the subterranean stem in response to death or removal of the apical meristem at the center of the crown of the cactus. The production and development of such lateral branches as a consequence of human harvesting of peyote is described and photographically documented by Terry and Mauseth (2006). Commercial peyote harvesting typically involves cutting the crown off at such a depth as to effect the removal of the apical meristem of the harvested plant. The lateral branches produced as a result (due to derepression of branching as a consequence of removal of the apical meristem, which is the source of branch-suppressant auxins) emerge from the ground as small crowns around the perimeter of the decapitated parent plant (Fig. 4), and eventually mature into independent plants that ultimately detach themselves from the degenerating body of the parent plant (Terry and Mauseth, 2006).

A problem arises in field interpretation of close clusters of crowns of *L. williamsii*. By definition a cespitose individual has a central “parent” stem, which has produced multiple lateral branches, each of which bears a crown, and each of these lateral branches in turn

may give rise to multiple lateral branches with their own crowns. Thus the key criterion for a cespitose plant is that all crowns are connected by living tissue (particularly vascular tissue) to the central parent stem; i.e., a cespitose plant is a single plant with multiple stem branches (Powell and Weedin, 2004). The problem lies in distinguishing a cespitose plant from a dense cluster of unconnected – but often contiguous – individual plants; such a cluster can be characterized as pseudocespitose. The mechanisms for the development of such pseudocespitose clusters of individual plants include the following:

- (1) The plants all germinated from seeds produced by a parent plant or plants, living or long dead. The adults that develop from such seeds are then simply separate individual plants that happened to germinate very close to the parent plant, and hence very close to each other, which is a frequent occurrence in cultivation, where *L. williamsii* is a copious seed producer (pers. obs.).
- (2) The crown of the original parent plant was harvested by humans who removed the apical meristem along with the crown (or “button”), whereupon lateral branching produced new crowns which put down their own adventitious taproots and ultimately became independent of the parent stem, which eventually degenerated and died (as in Fig. 4, where the harvesting occurred per the protocol of a controlled study of the effects of harvesting: Terry et al., unpublished data).
- (3) The crown of the original plant was killed, or the apical meristem was destroyed without necessarily killing the crown, by some environmental insult, such as undergoing severe frostbite and/or lyophilization as described above, or simply being stepped on by an ungulate. The progression of subsequent events would be essentially identical to those caused by harvesting – since the critical event is the loss of the apical meristem, regardless of the



specific cause of such loss – and the end result will likewise be the death and disappearance of the parent plant, leaving a dense cluster of independent clonal progeny occupying the spot where the parent plant had been.

This is not to imply that true cespitose *Lophophora* plants do not exist. On the contrary, in the early stages of the progression of events following loss of the apical meristem, every damaged parent plant undergoing the transition to a pseudocespitose cluster of independent clonal progeny plants must first go through a true cespitose phase, where the new crowns borne on lateral branches are not yet independent of the parent plant that lost its apical meristem. And if we broaden the taxonomic and geographic scope beyond *L. williamsii* in U.S. populations – and consider, for instance, the clump-forming habit of *L. fricii* growing in the Laguna de Viesca in Coahuila (Fig. 5; Terry, 2008a), *L. diffusa* in Querétaro (Fig. 6; Terry, 2008b), and indeed *L. williamsii* at Miquihuana, Tamaulipas (Fig. 7), and El Huizache, San Luis Potosí, Mexico (Fig. 8), as documented by Anderson (1969) and Terry (2008c and 2008b, respectively) – then we see what appear to be clear and impressive instances of cespitosity. However, the only way to be certain as to whether a multi-crowned *Lophophora* specimen (such as the specimen(s) from Terrell County, Texas, in Fig. 9) is cespitose or pseudocespitose, is to dig it up and look at the subterranean architecture of the plant(s) to see if the densely growing crowns are interdependent or if some are independent of the others. The destructiveness of such excavation, particularly in the case of what we infer to be very old clumps of cacti of vulnerable taxa, may be deemed too high a price to pay for scientific knowledge.

### ACKNOWLEDGEMENTS

I thank A. Michael Powell for valuable comments in his review of the manuscript. Root Gorelick provided a good excuse to get into the field to observe the effects of the hard freeze on the local cactus flora. Funding for this work was provided by a grant from the Alvin A. and Roberta E. Klein Foundation and a Research Enhancement grant from Sul Ross State University.



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Fig. 1. Typical healthy specimens of *Lophophora williamsii* in habitat in Presidio County, Texas.

Fig. 2a.

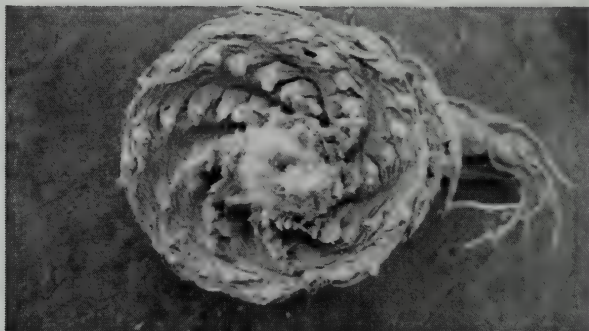


Fig. 2b.

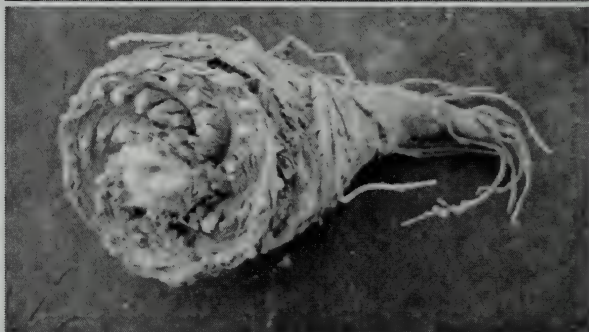
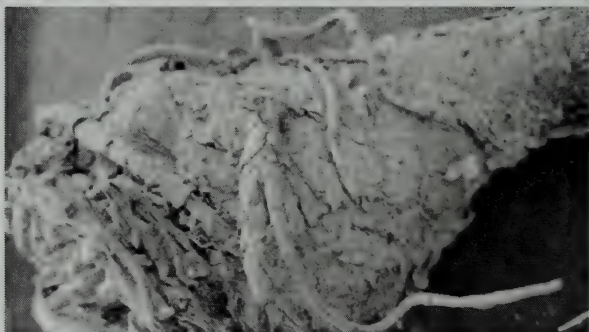


Fig. 2c.



Figs. 2a, 2b, and 2c. Specimen of *L. williamsii* whose crown underwent natural lyophilization in the cold, dry, somewhat high-altitude environment of the Big Bend region during the prolonged hard freeze of 2–5 Feb 2011.



Fig. 3. Four new offsets (“pups”) that emerged after transplanting the specimen shown in Fig. 1 to a pot in the greenhouse and providing water for 11 weeks. The emergence of offsets indicates that the plant is alive but its apical meristem is dead.



Fig. 4. Four offsets from a decapitated subterranean stem of a *L. williamsii* plant harvested in habitat three years previously in Jim Hogg County, Texas. Such regrowth in the form of lateral branches represents the typical response to harvesting in this species.





Fig. 5. A large clump of *L. fricii* growing in the Laguna de Viesca in Coahuila, Mexico.



Fig. 6. A typical clump of *L. diffusa* growing near a dry creekbed in Querétaro, Mexico.





Fig. 7. A clump of *L. williamsii* growing west of Miquihuana in southern Tamaulipas, Mexico.



Fig. 8. A relatively small clump of *L. williamsii* growing at El Huizache, in San Luis Potosí, Mexico.



Fig. 9. A clump of *L. williamsii* growing in Terrell County, Texas. The question goes begging as to whether it is a cespitose individual or a pseudocespitose cluster of individuals.

Note: The photographs for Figures 1-9 can be viewed in color at:  
<http://www.cactusconservation.org/CCI/Lwfreezedry.html>

## TAXONOMY AND DISTRIBUTION OF *SENECIO PARRYI* (ASTERACEAE)

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### ABSTRACT

**Senecio parryi** A. Gray is typified by material collected in north-central Mexico, presumably in northwestern Coahuila, just across the border from Texas along the Rio Grande. It is a relatively rare species in the area where first collected, but has subsequently been collected in the USA (New Mexico and Arizona) and northeastern Mexico (Coahuila, Chihuahua, Sonora, and northern Sinaloa). Two named taxa (*S. pringlei* A. Gray from Chihuahua and *S. ritovegana* B.L. Turner from northern Sinaloa) are reduced to synonymy under its fabric. A distribution map of the complex is provided, along with rationale for the synonymy indicated. *Phytologia* 93(3): 341-345 (December 1, 2011).

**KEY WORDS:** Asteraceae, *Senecio*, *S. parryi*, *S. pringlei*, *S. ritovegana*

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*Senecio parryi* is a very distinct taxon, this not positioned as to "Group" by Barkley (2006) in his treatment of North American *Senecio*, but rather left in limbo among the "Exceptional" species." Still, it is superficially similar to *S. multidentatus* and *S. lemmonii* of Arizona, but readily distinguished by its glandular pubescent foliage and pubescent achenes. Parry, in 1850, first collected the species along the Rio Grande, in the Big Bend region of Texas, along the Mexican side. It was subsequently collected in Arizona by yet others, where relatively common, and only once in Chihuahua by Pringle in 1886, where given the name *S. pringlei* A. Gray. Numerous recent collections of the taxon have been made in Sonora, Mexico, reevaluation of which has led to the submergence of *S. ritovega*, into



the newly conceived, broad circumscription, of *S. parryi*. The taxonomy of the complex follows:

**SENECIO PARRYI** A. Gray, Rep. U.S. Mex. Bound. Surv. 2: 103.

1859. TYPE: **MEXICO. Coahuila**. "In live-oak groves, 150 miles above the mouth of the Pecos, on the Mexican side of the Rio Grande, November;" *Parry s.n.* (holotype : GH).

*Senecio pringlei* A. Gray, Proc. Amer. Acad. Arts 22: 307. 1887. **Fig. 1.**

TYPE: **MEXICO. Chihuahua**. Shaded places, Mapula Mountains, Oct 1886, *C.G. Pringle 763* (holotype: GH; isotype LL!).

*Senecio ritovegana* B.L. Turner, Phytologia 80: 95. 1996. **Fig. 2.**

TYPE: **MEXICO. Sinaloa**. Mpio. Badiraguato: a 15 km al N. de Surutato rumbo a Sta. Rita, 2000-2200 m, 9 Dec 1987, *Rito Vega 2550* (holotype: TEX!)

**Annual or short-lived perennial herbs** to 8 dm high; leaves and stems viscid-pubescent with crisped glutinous-glandular hairs or short, glandular trichomes, the herbage with a distinctive odor. **Stems** branching mostly in the upper third, arising singly from a tap-root. **Leaves:** lower and middle cauline mostly lanceolate, tapering to a winged petiole with an expanded, clasping base, margins variously and irregularly sharp-dentate; well-developed leaves 8-12 cm long, 2-5 cm wide, reduced and auriculate-clasping distally. **Capitulescence** an open corymbiform cyme of 12-30 heads; involucre bracts ca 21, 7-9 mm long; calyculus of linear bracts 3-8 mm long. **Ray florets** ca 13, the ligules yellow, mostly 8-12 mm long. **Achenes** densely appressed-pubescent; pappus of numerous, white deciduous bristles. **Distribution:** see Figure 3.

In a preliminary draft of the Mexican species of *Senecio sensu lato*, Barkley and Turner (ca 1990) placed *S. pringlei* in synonymy with the present taxon, this reaffirmed herein.

At the time of my description of the Sinaloan *Senecio ritovegana*, relatively few collections of *S. parryi* were known from Sonora, Mexico. Recent collections from the latter region, mostly by Tom Van Devender, have shown that the characters by which I distinguished *S. ritovegana* (mainly vestiture and elongate calycular



bracts) are quite variable in the region concerned, and I have little hesitancy in reducing this to synonymy here.



Figure 1. Type of *Senecio pringlei* A. Gray.

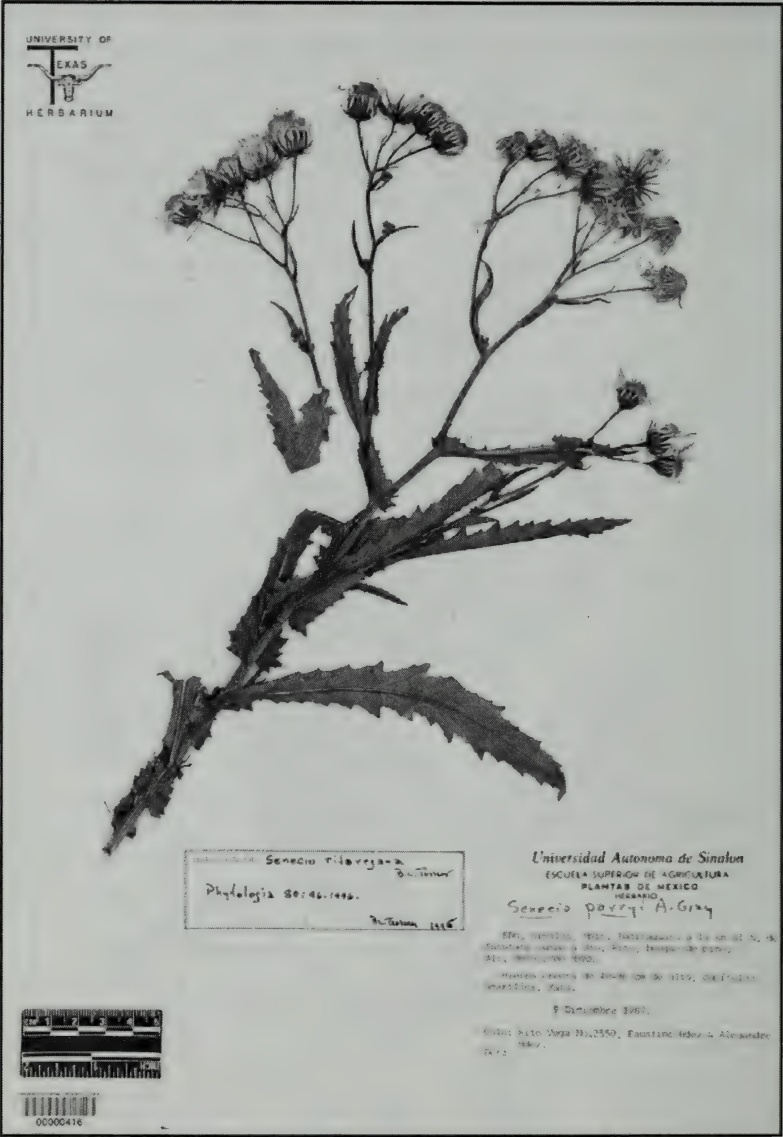


Figure 2. Type specimen of *Senecio ritovegana* B. L. Turner.

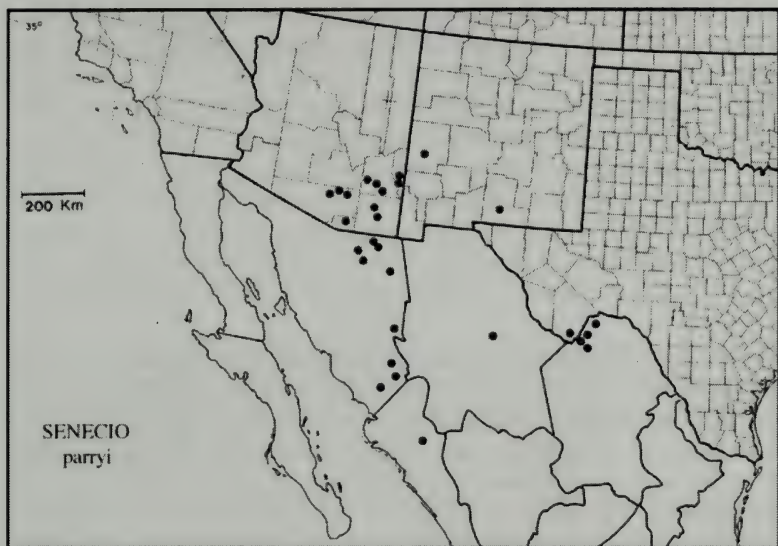


Figure 3. Distribution of *Senecio parryi*.

### ACKNOWLEDGEMENTS

My colleague Dr Guy Nesom kindly read the paper and made helpful suggestions. Thanks to the following herbaria for the loan of specimens, upon which the distribution map (Fig. 3) is based: ARIZ, ASU, and NMU.

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Barkley, T.M. 2006. *Senecio*, in Fl. North America 20: 215-570.

A NEW SPECIES OF *DECACHAETA* (ASTERACEAE:  
EUPATORIEAE), FROM OAXACA, MEXICO

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ABSTRACT

A new species, *Decachaeta serboana* B.L. Turner, **sp. nov.** is described from Oaxaca Mexico. It is closely related to the more wide spread *D. incompta*. A photograph of the type is provided, along with a revised key to the Mexican taxa. A map showing the distribution of *D. incompta* and *D. serboana* is presented. *Phytologia* 93(3):346-350 (December 1, 2011)

**KEY WORDS:** Asteraceae, Eupatorieae, *Decachaeta*, *D. incompta*, Mexico, Oaxaca.

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Routine identification of Mexican comps has prompted the present paper.

**DECACHAETA SERBOANA** B.L. Turner, **sp. nov.** Fig. 1

*Decachaetae incomptae* (DC.) King & H. Rob. similis sed differt foliis tenuioribus glabris, capitulis minoribus (2-3 mm in altitudine vs. 4-5 mm), flosculis paucioribus (3-5 vs. 8 vel plus), et receptaculis glabris (vs. pubescentibus).

**Perennial herbs** or straggling shrublets to 1.5 m high. **Stems** glabrate or nearly so, straight to somewhat fractiflex above. **Leaves** alternate, thin, ovate-lanceolate to somewhat deltoid-lanceolate, 10-30 cm long, 7-18 cm wide; petioles 1-10 mm long; blades pinnately nervate, glabrous above and below, the lower surfaces minutely punctate, their margins markedly dentate-lacerate. **Capitulescence** a terminal,



bracteate, cymose panicle 15-30 cm long, 15-25 cm wide, the ultimate peduncles 1-2 mm long, viscid-puberulent. **Heads** narrowly campanulate, 3-4 mm long, ca 1.5 mm wide. **Receptacles** ca 0.5 mm across, epaleate, glabrous or sparsely pubescent. **Involucres** 2-3 mm long, ca 1.5 mm wide, markedly atomiferous-glandular to nearly glabrate; bracts ca 8, 2-4 seriate, gradate, their apices broadly acute to obtuse. **Florets** 3-4(5) per head; corollas white, 5-lobed, ca 2 mm long, the throats indistinct. **Stamens** with terminal appendages broader than long. **Achenes**, ca 1 mm long, 4-sided, sparingly hispid with ascending hairs; pappus of ca 20-25 setaceous bristles 1.5-2.0 mm long.

TYPE: MEXICO. OAXACA: **Mpio. Santiago Textitlan**, "sur de Tierra y Libertad camino a Zaniza," pine-oak forests, ca 1830 m, 15 Nov 2006, *Silvia H. Salas M. 6086* [con A. Nava y A. Sanchez] (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: MEXICO. OAXACA: **Mpio. San Juan Lachao**, 34.5 km N of Mixtepec, on road from Puerto Escondido to Oaxaca. "Half-shaded, wet slope along a stream," 1850 m, 13 Nov 1997, *Yahara et al. 1130* (TEX). **Mpio Santa Cruz Itundujia**, "La Estaca, sobre brecha, a 1.66 km en LR (N) de la Agencia de Morelos," ca 1500 m, 11 Oct 2007, *Gutierrez 2430* (TEX). **Mpio. Santiago Textitlan**, "Paraje arriba de Rio Tronco rumbo a Rio Palo," cut-over pine-oak forests in red soil at mouth of arroyo, ca 1781 m, 4 Nov 2006, *Olazo 816* (TEX); "Paraje Rio Aguacate," ca 1875 m, 14 Dec 2006, *Olazo 1123*; "Arriba de Barranca Nube," pine-oak forests (16 41 38 N, 97 15.5 01W), ca 1891 m, 27 Dec 2006, *Salinas 1322* (TEX). **Mpio. Santiago Juchitahuaca**, "Senda para el rio y sacada de san Juan Pinas." ca 1375 m, 22 Nov 1995, *Calzada 20506* (TEX).

In my treatment of *Decachaeta* for The Comps of Mexico (Turner 1997), *D. serboana* will key to *D. incompta* (DC.) King & H. Rob., the latter readily distinguished by its larger, usually markedly pubescent leaves, and larger heads having more numerous florets. Distribution of the two species is shown in Fig. 2.

The species name is an acronym of the Sociada para el Estudio de los Recursos Bioticos de Oaxaca (SERBO). This organization has helped fund the collection of numerous plants from the area concerned.

A revised key to the Mexican species of *Decachaeta*, with the addition of the present novelty, follows:

1. Leaves along upper stems alternate.....(2)
1. Leaves opposite throughout.....**D. perornata**
2. Leaves not glandular-punctate beneath.....**D. haenkeana**
2. Leaves clearly glandular-punctate or atomiferous-glandular beneath.....(3)
3. Petioles winged throughout.....(6)
3. Petioles without wings.....(4)
4. Pappus a ring or crown of minute bristles 0.2 mm long or less; receptacle plane, glabrous or nearly so.....**D. pyramidalis**
4. Pappus of bristles 2-5 mm long; receptacle convex, pubescent.....(5)
5. Involucral bracts 12-15; florets 10-12 per head.....**D. ovatifolia**
5. Involucral bracts 6-10; florets 6-10 per head.....**D. scabrella**
- 6(3). Phyllaries narrowly acute; corollas lavender; Cps...**D. ovandensis**
6. Phyllaries acute to rounded; corollas white to pinkish.....(7)
7. Leaves pubescent beneath (rarely not); involucre 4-5 mm long; widespread.....**D. incompta**
7. Leaves glabrous beneath; involucre 2-3 mm long; sw Oax.....  
.....**D. serboana**

### ACKNOWLEDGEMENTS

I am grateful, as usual, to my long-time colleague, Guy Nesom, for the Latin diagnosis and reviewing the paper. The map (Fig.2) showing distributions is based upon specimens on file at LL-TEX.

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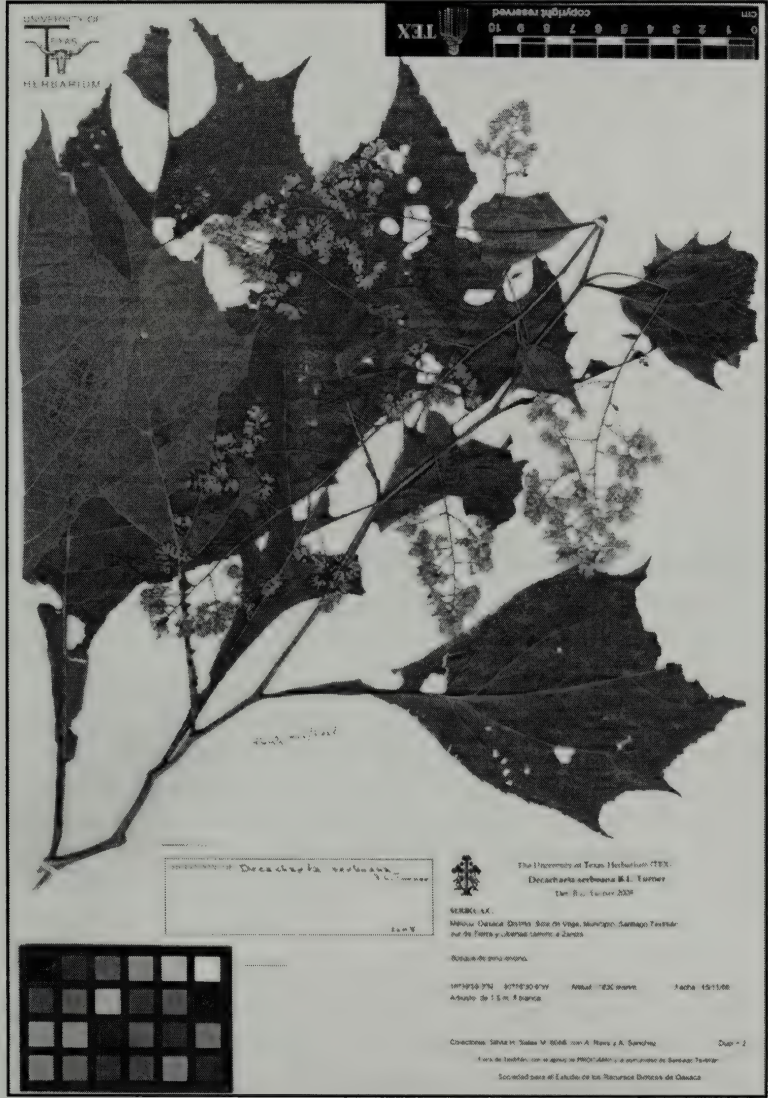


Fig. 1. Holotype of *Decachaeta serboana*.

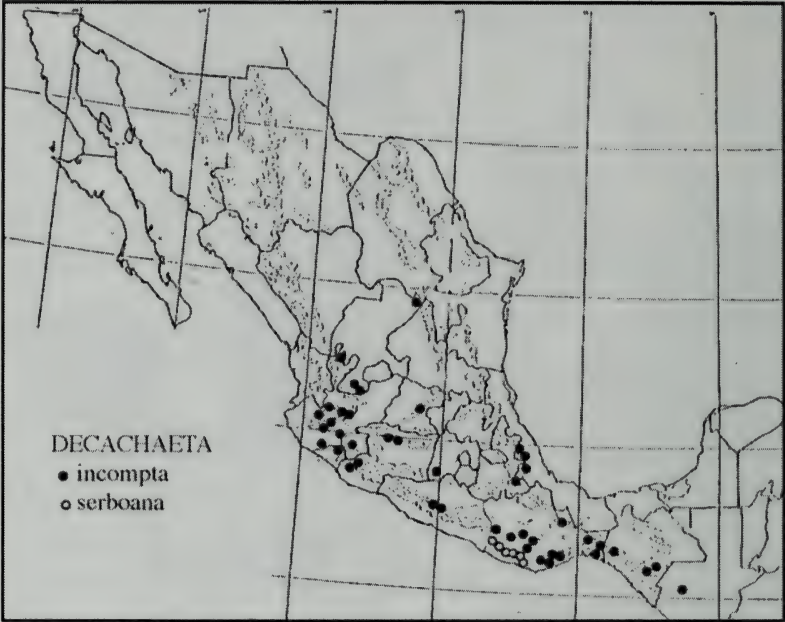


Fig. 2. Distribution of *Decachaeta incompta* and *D. serboana*.



## DNA FROM HERBARIUM SPECIMENS: II. CORRELATION OF DNA DEGRADATION WITH HUMIDITY

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### ABSTRACT

Comparisons were made of DNA extracted from leaves of *Juniperus virginiana* stored in 100, 75, 70, 54 and 23% humidity at 21°C for up to 12 months. Fungal growth was observed in the 100, 75 and 70% humidity tests that resulted in the degradation of the juniper DNA. The DNA in leaves stored at 54.4 and 23.1% RH show no evidence of degradation after 12 months storage. It appears that storage of herbarium specimens at sub-ambient temperatures and at RH less than 55% should be effective in preserving DNA *in situ*. *Phytologia* 92(3): 351-359 (December 1, 2010).

**KEY WORDS:** DNA, herbarium specimens, humidity, degradation, *Juniperus*.

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Recently, Adams and Sharma (2010) reported on DNA extracted from *Juniperus* herbarium specimens ranging from 1 to 80 years old. They found the size of DNA declined with age, but varied considerably for specimens less than 20 yrs. old. After about 20 yr. the size of the DNA appeared to asymptote at about 200 - 500 bp. They concluded that variation in the quality of DNA from recent specimens may be due to drying methods and storage conditions (humidity, temperature).

It is now standard procedure to collect specimens and put some leaves in silica gel for subsequent DNA extraction. Liston et al. (1990) published the first paper that utilized silica gel in the field, although Doyle and Doyle (1987) suggested that drying appeared to be effective in preserving DNA. Pyle and Adams (1989) reported that spinach that was desiccated in Drierite® (anhydrous CaSO<sub>4</sub>), then

stored under ambient herbarium conditions yielded excellent DNA for up to 2 months, but their next (5 mo.) sample displayed some DNA degradation. Liston et al. (1990) reported that spinach stored in silica gel at 21°C showed very little degradation after 5 months.

Telle and Thines (2008) reviewed the extraction of DNA and amplification of *cox2* from herbarium specimens. They note that despite success in the utilization of animal remains and even coprolites from the Miocene, it is still a major challenge to obtain DNA from many herbarium specimens. They attribute this to suboptimal drying and storage conditions. Telle and Thines (2008) reported large differences in the efficiency of different extraction methods and various DNA polymerases used to amplify *cox2*.

The maintenance of constant humidity in laboratory chambers can be easily achieved by the use of saturated salt solutions (Young, 1967; Greenspan, 1977). Table 1 shows a number of salts that give a useful range of humidities.

Table 1. Saturated salt solutions useful for maintaining a certain level of humidity (values at 20°C). Adapted from Young (1967) and Greenspan (1977). Salts used in this study are in boldface.

salt	% humidity	cost/g	sat. soln.		rating
			g/100ml	\$/100ml	
lithium bromide	6.61	\$1.60	160	\$256	*
zinc bromide	7.94	4.46	446	1989	--
lithium chloride	11.3	0.84	83.5	70	**
lithium Iodide	18.56	2.70	165	445	-
<b>potassium acetate</b>	<b>23.11</b>	<b>0.05</b>	<b>256</b>	<b>12</b>	***
magnesium chloride	33.07	0.015	54.6	0.8	***
potassium carbonate	43.16	0.10	111	11	***
<b>magnesium nitrate</b>	<b>54.38</b>	<b>0.13</b>	<b>69.5</b>	<b>9</b>	***
<b>potassium iodide</b>	<b>69.90</b>	<b>0.19</b>	<b>144</b>	<b>27</b>	**
<b>sodium chloride</b>	<b>75.47</b>	<b>0.01</b>	<b>35</b>	<b>0.4</b>	***
<b>water</b>	<b>100.0</b>	<b>nil</b>	<b>--</b>	<b>--</b>	***

The purpose of this study was to examine the effects of humidity on the stability of DNA in *Juniperus virginiana* leaves to gain a better understanding about the degradation of DNA in herbarium specimens.

## MATERIALS AND METHODS

Plant specimen utilized: *Juniperus virginiana* L., Adams 12286, cultivated, Gruver, TX. Specimen deposited at BAYLU.

Fresh leaves of *J. virginiana* were air dried for 24 h in a plant press at 40°C. Then the leaves were thoroughly mixed and a random sample of 5 g of leaves was placed in an aluminum weighing dish and thence into a plastic container that was then sealed (Fig. 1). The hygrometer inside the jar was used to monitor humidity. The excess salt can be seen in the saturated solution the bottom of the jar (Fig. 1).

After intervals of 1, 2, 3, 4, 6, 9, and 12 months, leaves were removed for analyses.

DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

Genomic DNA was visualized by agarose gel electrophoresis by mixing 4 µl DNA extract plus 1 µl loading buffer and run

on a 1.5% agarose gel, at 70 v for 55 min. The DNA size marker consisted of 3 µl pGEM markers and 3 µl λHindIII, with 6 µl loaded onto the gel. The images were captured on a Kodak Gel Logic 100

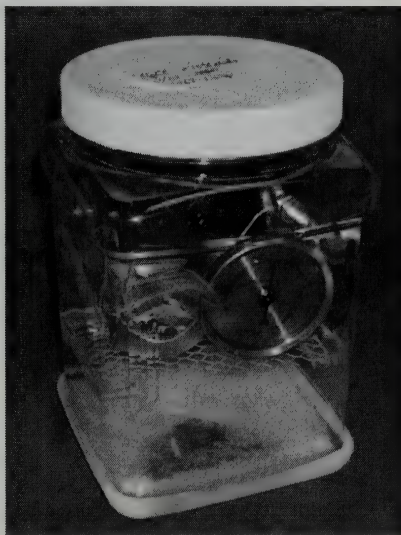


Figure 1. Sealed container with *J. virginiana* leaves suspended above saturated salt solution.

Imaging System, and profile analysis was used to determine the modal DNA size and range of DNA sizes. The DNA from some samples was subjected to PCR amplification. ITS (nrDNA) amplifications were performed in 30  $\mu$ l reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15  $\mu$ l 2x buffer E (petN-psbM) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200  $\mu$ M each dNTP, plus Epi-Centre enhancers with 1.5 - 3.5 mM  $MgCl_2$  according to the buffer used) 1.8  $\mu$ M each primer. See Adams, Bartel and Price (2009) for the ITS primers utilized.

RESULTS

In general, there was a rapid decline in the genomic DNA in the 100% RH test with a more gradual decline in the 75% and 69.6% RH tests (Fig. 2). At 2 mos. storage there are noticeable breakdown products in the 100% RH test and some loss of the genomic DNA in the 75% RH chamber (Fig. 2). At 6 mos., much of the genomic DNA is

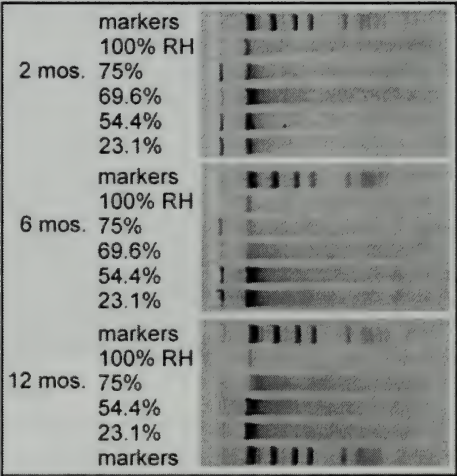


Figure 2. Gels of DNA from 2, 6, and 12 months storage at various humidities. The size marker lanes are  $\lambda$ /HindIII + pGEM.



degraded in the 75% and 69.6% RH tests. After 12 months, only the DNA from 54.4 and 23.1% RH tests appears intact.

Within a few days, a filamentous fungus appeared on the leaves in the 100% RH chamber (Fig. 3). The fungus continued to grow for the next several months. Due to the growth of the fungus, the DNA from the 100% RH chamber maintained a sharp 'genomic' band of fungal DNA (Fig. 2). The DNA from the 100% RH was prepped and the nrDNA region sequenced. A BLAST search in GenBank gave the highest match (67%) to an endophytic fungus in lichen (HM123443). It seems likely that juniper has an endophytic fungus that is not killed by leaf drying. The fungus started to grow in a few days in the 100% RH chamber (Fig. 3).

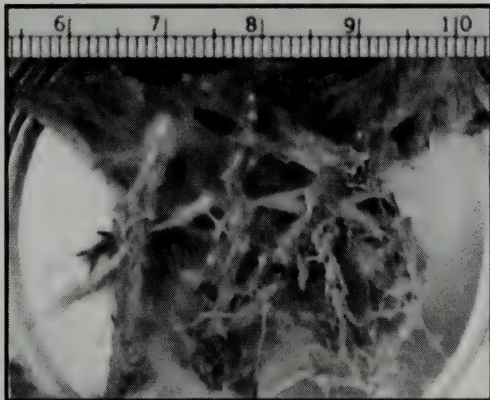


Figure 3. Juniper leaves covered by a white filamentous fungus in 100% RH chamber.

Profile analyses of the DNA after leaf storage for 2 months reveals the breakdown in the 100% RH test and some breakdown in the 75% RH test (Fig. 4). Storage at 69.9, 54.4, and 23.1% RH show very little breakdown after 2 months.

Profile analyses of the DNA after leaf storage for 12 months shows extensive breakdown in the 100 and 75% RH (Fig. 5). Note the sharp genomic peak in the 100% RH test. This is the fungal genomic DNA, as the juniper genomic DNA has been degraded by this time. The genomic peak in the 75% RH test (Fig. 6) is likely fungal DNA as fungal growth was also detected in this test.

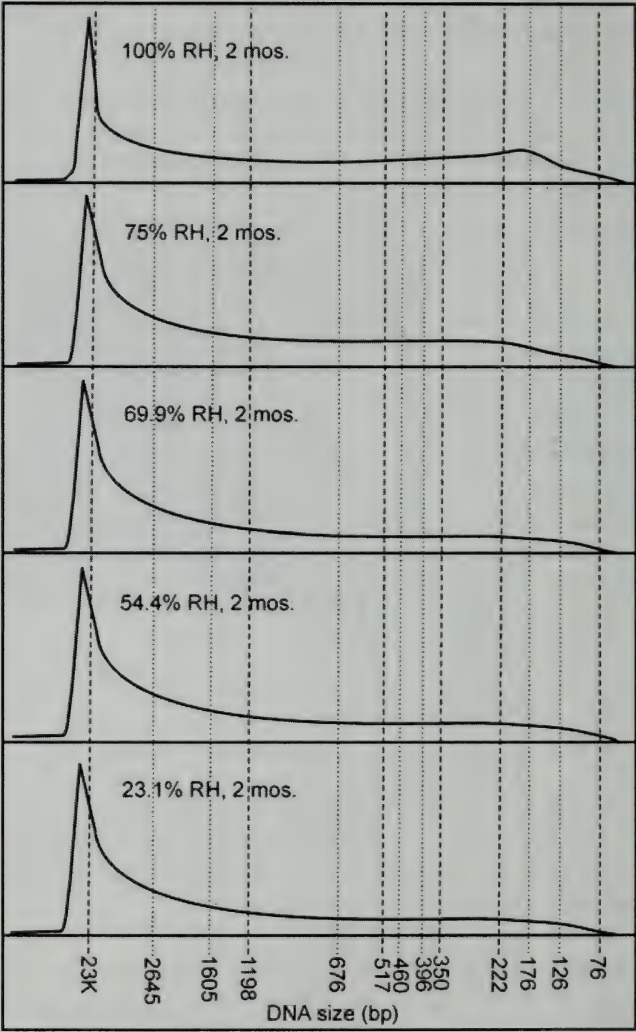


Figure 4. Profile analysis of *J. virginiana* DNA from leaves stored for 2 months at various humidity levels.

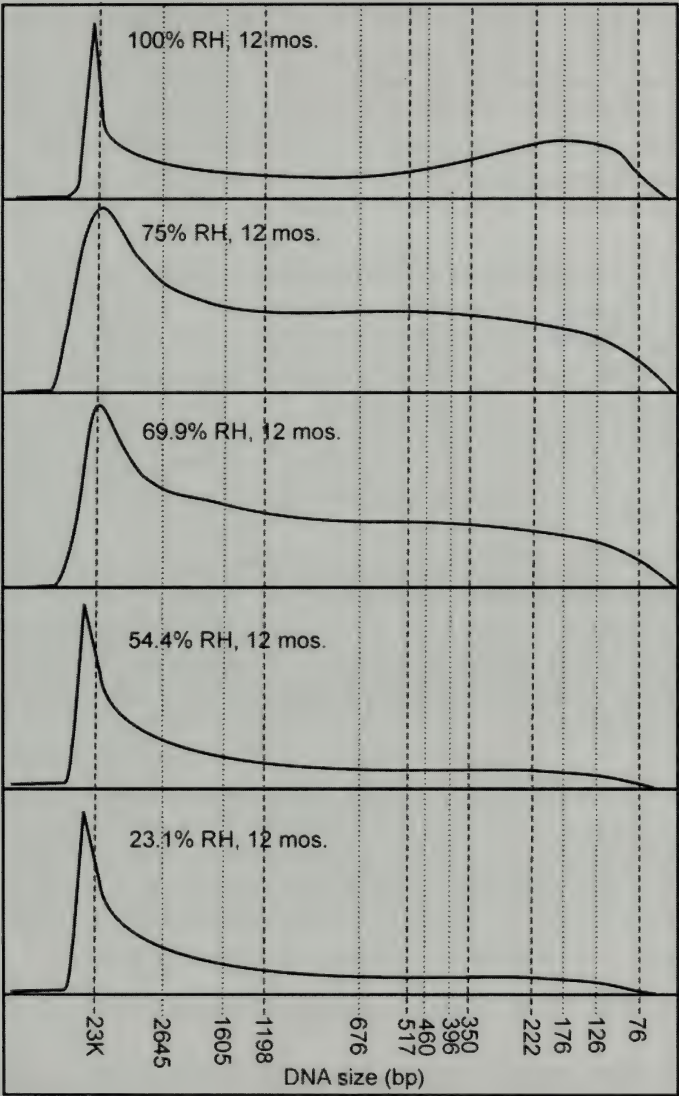


Figure 5. Profile analysis of *J. virginiana* DNA from leaves stored for 12 months at various humidity levels.

The leaves stored at 75 and 69.9% RH are showing some degradation after 12 mos. at RT. Notice the shift of the modal peak to about 23 Kb bp and the more rounded nature of the modal peak (Fig. 5) as well as the long tailing of degraded DNA from 23 Kb down to 76 bp. However, the leaves stored at 54.4 and 23.1% RH yielded genomic DNA with very little breakdown from 2 mo. (Fig. 2) to 12 mo. storage (Fig. 12).

It appears, in this study, that the growth of fungus was the main cause in the degradation of juniper DNA. Fungal growth appears to be inhibited at RH of ~55% or less. Viitanen (1994) reported that the growth of fungi on wood was inhibited at RH less than 80%. They also found that at lower temperatures (eg., 5°C), fungi would only grow at higher RH (eg. 87 - 90 % RH). Nielsen et al. (2004) found that the lower limit for fungal growth on wood and starch-containing composites was 78% RH at 20-25°C and greater than 90% RH at 5°C.

Block (1953) compared mold growth on a variety of substrates (leather, cotton, wood, wool, cheese and glass wool) at various relative humidities at 85°F (29.4°C). He concluded that these materials could be stored free of mildew at 65% RH. However, for the internal fungi found in plants, it seems likely that internal fungi are more robust than mildew.

Our herbarium (BAYLU) is maintained at 60°F and 40% RH to minimize fungi and insect growth. It appears that herbarium specimens stored at ~55% relative humidity should inhibit fungal growth and maintain well-preserved DNA. Unfortunately, many (most?) specimens in herbaria have been exposed to much higher humidity levels before air conditioning was widely utilized.

### ACKNOWLEDGEMENTS

Thanks to Tonya Yanke for lab assistance. This research was supported, in part, with funds from Baylor University.



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## THE HISTORICAL STABILITY OF NEVADA'S PINYON-JUNIPER FOREST

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### ABSTRACT

The singleleaf pinyon-Utah juniper forests of Nevada have long been depicted as invasive communities that have expanded from sparse populations on rough terrain to overwhelm large areas of shrubland, reducing their forage value. This paradigm has led to deforestation programs to restore a cover condition thought by range managers to have characterized these lands at the time of settlement in the mid-19th century. We examine contemporary descriptions of the forest, mainly germane to the immense wood resources needed to support the mining and smelting industry. The early descriptions indicate that the pinyon-juniper forest was widespread, continuous over many mountain ranges throughout much of the state, and frequently dense. A comparison of lower forest border elevations reported in the 19<sup>th</sup> century with those currently mapped show no evidence of downward expansion. Three case studies of areas documented to have been deforested in the 19<sup>th</sup> century, have naturally re-forested, showing the resilience of the forest. Deforestation for restoration reasons is not justified in the absence of site-specific evidence that shrubland invasion has occurred in historic times. *Phytologia* 93(3): 360-387 (December 1, 2011)

**KEY WORDS:** pinyon-juniper, singleleaf pinyon, Utah juniper, woodland expansion, invasive plants, John Muir, Nevada forests, Great Basin ecology

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*The vestiges of a once-flourishing wood products industry haunt the current managers of the pinyon-juniper zone* – J. A. Young and J. D. Budy, 1979

The pinyon-juniper forest of singleleaf pinyon (*Pinus monophylla* Torr.& Frém.) and Utah juniper (*Juniperus osteosperma* [Torr.] Little) is widespread in Great Basin portions of Utah and Nevada. It has been estimated at 7.6 million acres in Nevada (Miles 2011), with the vast majority on public lands. The small size and slow growth of these drought-adapted trees have long presented a utilization conundrum to the USDA Forest Service and USDI Bureau of Land Management. As a result, commodity production has stressed livestock rather than forest products.

Starting in the 1950s, fears were expressed within the managing agencies that the pinyon-juniper forest was rapidly expanding – by invading previously treeless lands and by becoming denser -- thus threatening the productivity of historical rangelands whose forage plants could not compete with the conifers. The management response was to deforest lands presumed to have been so invaded, and convert them to pasturage for cattle (USDA Forest Service 1973, 1974). Between 1960 and 1972, trees on over a third of a million acres in Utah and Nevada were uprooted by chains dragged behind bulldozers (Lanner 1981). Later plans were to deforest almost 400,000 more acres in those states (USDA Forest Service 1973, 1974). “Chaining” abated during the 1970s and 1980s, in part because benefit-cost ratios for enhanced forage production proved generally negative (Workman and Kienast 1975, Clary 1989). Further, range scientists reported that cleared areas often began to reforest naturally within 15 years (Tausch and Tueller 1977), raising questions about the permanence of deforestation (Lanner 1977, 1981).

A core issue regarding this pinyon-juniper forest, and other species combinations elsewhere in the West, has been the cause of its expansion. For several decades it has been suggested that grazing, fire exclusion, or climate change have been responsible, yet “...surprisingly little empirical or experimental evidence is available to support or refute any of these hypotheses; most interpretations are based on logical inference” (Romme et al. 2009). Nor has more than passing notice

been given the impacts of forest clearing for fuel wood, charcoal manufacture, posts, ties and structural timber during the settlement and silver mining era in 19<sup>th</sup> and early 20<sup>th</sup> century Nevada, despite the magnitude of those impacts having been frequently described (Lanner 1977, 1981, Young and Budy 1979, Young and Svejcar 1999, Charlet 2008, Straka and Wynn 2008). Even the frequently cited “invasion paper” by Blackburn and Tueller (1970) makes no mention of past harvest, despite the study areas being located between and among important 19<sup>th</sup> century mining centers.

Another key issue is the magnitude of expansion. A view that has become influential in range science is that much of the pinyon-juniper forest was savanna-like – a shrubland or grassland with scattered trees – in which old trees were restricted to fireproof rocky or dissected topography (West 1988).

This led to the “logical inference” that the present area of forest has resulted from aggressively invasive behavior since settlement, more than doubling the pre-settlement area (Miller et al. 2008), and perhaps increasing it ten-fold (Miller and Tausch 2001). The first of these estimates is based on demographic studies conducted on one of the 126 Nevada mountain ranges (0.8 %) upon which singleleaf pinyon grows (Fig. 1), the forest history of which was barely considered; and a similar study in one central Utah range. The second estimate conflates all western North American woodlands, so is not discussed further. Some estimates confound expansion into unforested areas with increased density of existing forest (“infill”), so “invaded” acreages may have limited meaning (Romme et al. 2009, Weisberg et al. 2007).

Recent field and modeling studies in the Simpson Park Range of central Nevada, known to have been harvested for charcoal production, show that evidence of mining-era forest harvest and re-growth can be detected if appropriate forensic methods are employed (Ko et al. 2011). This work was facilitated by historical data that identified areas likely to have been harvested, and the location of transportation networks. In principle, such harvest evidence should be discoverable anywhere deforestation has occurred, using these techniques and realistic assumptions of stump and relic decay rates



(Reno 1994, Wessels 2010, Ko et al. 2011). Strachan (2011) has subjected even carbonized remnants and cultural remains of both juniper and pinyon to careful dendrochronological analysis, including use of local tree-ring chronologies.

## OBJECTIVES

In this article we consult historical records of pinyon-juniper forest distribution and abundance in 19<sup>th</sup> century Nevada for evidence that supports or refutes the hypothesized large-scale invasion of shrublands. This task is made more difficult – and, paradoxically–made easier, by the history of land use particular to Nevada. On the one hand, the original 19<sup>th</sup> century forest was very heavily impacted by the mining boom that began in western Nevada's Comstock Lode in 1859, and continued with interruptions throughout the state into the early 1900s. So today's forest is significantly modified from what would have been its natural trajectory. On the other hand, the critical importance of fuel wood to the mining industry fostered an unprecedented level of documentation by agents of the State of Nevada, and the United States, of the industrial potential of pinyon and juniper fuel wood.

## METHODS

For information on the distribution, occurrence and characteristics of singleleaf pinyon-Utah juniper forest in 19<sup>th</sup> century Nevada, we consulted official contemporary documents of the State of Nevada and the United States Government that resulted from explorations and on-site assessments of mining; and the writings of John Muir, Charles Sprague Sargent, E. W. De Knight, Franklin B. Hough, C. Hart Merriam and Frederick V. Coville. Data on present-day distribution of singleleaf pinyon were taken from *Nevada Conifers, A Phytogeographic Reference* (Charlet 1996). This reference was also used for the names of most mountain ranges, several of which have changed since the 19<sup>th</sup> century.

Present-day elevations of forest borders and the names of several mountain ranges were taken from topographic maps in *Nevada Atlas & Gazetteer* (DeLorme 2008). A mountain range base was

identified by locating the abrupt change in spacing of contours (interval = 200 ft.) found where the steep mountain or hill slope continues on a gentler gradient into the nearly flat valley below, usually termed the pediment or bajada. This allows comparison with the elevation of the border of woodland shading (mean of two locations on east slope, and two on west slope). According to DeLorme (personal communication, customer service representative, June 2011) the DeLorme topographic maps are subjected to field testing with regard to woodland shading. Singleleaf pinyon, and by definition the pinyon-juniper forest or woodland, is almost entirely absent from the wedge-shaped area of the state north of the Humboldt and Truckee Rivers (Charlet 1996), so few relevant data were found there.

## RESULTS

### PINYON-JUNIPER FOREST DISTRIBUTION IN 19<sup>TH</sup> CENTURY NEVADA

#### **Geographic Distribution**

The occurrence of singleleaf pinyon- Utah juniper forests in Nevada at the time of settlement or shortly thereafter is documented in contemporary accounts at three geographic levels – that of the macro-landscape, the individual mountain range, and the Mining District. Singleleaf pinyon is most often referred to in contemporary documents as “nut pine”, in recognition of the food value of its large nut-like seeds to the Indian inhabitants of the Great Basin (Lanner 1981). Utah juniper is referred to as “cedar”, “mountain cedar”, or “juniper”. When the species occur together in Nevada, singleleaf pinyon is usually dominant or codominant (Charlet 1996).

#### 1. Contemporary Macro-Landscape Observations

Referring broadly to Nevada’s mountains, Browne and Taylor (1867) wrote “They are covered nearly everywhere from base to summit with a growth of terebinthine (i.e. resinous) forests, consisting of a variety of pine....” Base elevation was put at 5,000 ft., summit elevation at 9,000 ft.

That same year, Stretch (1867) reported on an exploration made the spring of the preceding year in southern Nevada by Governor

Blaisdel. Along the route from Indian Springs to Pahrnagat, a four to five-day trip,” It will be seen that the whole of this section of the State is tolerably well supplied with wood and water.”

Two years later, Stretch (1869) reported that “The nut pine, the juniper and the mountain mahogany (*Cercocarpus ledifolius* Nutt.), thinly cover portions of the mountains through the interior and western sections of the State....When it is stated that such timber is abundant, it is only meant as a temporary supply (as it) can never be abundant as the pine in the forests of the Sierras....” Stretch’s apparent low appreciation of the pinyon-juniper forest resource is at variance with his frequent use of such terms as “dense”, “abundant”, “covered for miles”, and even “inexhaustible” when describing specific locations (see below).

Reporting on his explorations of 1871, Lt. Wheeler (1872) wrote: “Piñon pine and a stunted growth of mountain cedar abound in frequent localities in Nevada”.

The most detailed early observations of large scale singleleaf pinyon occurrence were those of John Muir. In the summer of 1878 the naturalist accompanied a U. S. Coast and Geodetic Survey triangulation party in a “rambling mountaineering journey of eighteen hundred miles” across and within Nevada (Badé 1924). Muir ascended the Augusta Mountains, the Desatoya Range and the Shoshone Mountains. He crossed the Reese River Valley, climbed the Toiyabe Range, and arrived in the mining center of Austin. He traveled down Big Smoky Valley, climbed the Toiyabe Range a second time as well as the Toquima Range, went south to Lone Mountain and climbed it, ascended the Hot Creek Range and traveled to the mining center of Belmont. He journeyed to another mining center, Hamilton, climbed Mt. Hamilton in the White Pine Range and visited a fourth mining center, Treasure City. His itinerary also included Ward, still another center of mining, in the Egan Range, and finally the Snake Range, which he apparently climbed. In addition, it is clear that he also climbed the Golden Gate Range, as he encountered Great Basin bristlecone pine (*P. longaeva* D. K. Bailey) there (Muir 1961). While resting later in the smelting hub of Eureka, the smoky “Pittsburgh of the West”, he wrote the essay “Nevada Forests” which later appeared as Chapter 13 of *Steep Trails* (Muir 1918).



Thus, Muir had first-hand knowledge of at least eleven Nevada mountain ranges, and ascended at least ten of them. Of the singleleaf pinyon, or nut pine, he generalized: "In the number of individual trees and extent of range this curious little conifer surpasses all the others combined. Nearly every mountain in the State is planted with it, from near the base to a height of from eight thousand to nine thousand feet above the sea. Some are covered from base to summit by this one species, with only a sparse growth of juniper on the lower slopes to break the continuity of these curious woods....Tens of thousands of acres occur in one continuous belt. Indeed, the entire State seems to be pretty evenly divided into mountain ranges covered with nut pines and plains covered with sage - now a swath of pines stretching from north to south, now a swath of sage; the one black, the other gray; one severely level, the other sweeping on complacently over ridge and valley and lofty crowning dome."

Muir saw the inroads that mining and settlement were making in these forests, and commented that "Many a square mile has already been denuded in supplying these demands, but so great is the area covered by it, no appreciable loss has as yet been sustained."

Muir observed that "... you find the ground beneath the trees, and in the openings also, nearly naked....Here and there occurs a bunch of sage or linosyris, or a purple aster, or a tuft of dry bunch-grass".

Following a "hurried journey "to Nevada, famed dendrologist C. S. Sargent (1879) remarked that at first the landscape seen from the new Pacific Railroad appeared almost destitute of trees. "The first impression will disappear, however, should (the traveler) penetrate further south, and ascend some of the low mountain ranges...." where "Large areas of forest-covered mountain ranges are still held by the General Government...." The railroad was routed along the Humboldt River, the northern boundary of singleleaf pinyon across much of the state.

Clearly, the extent of pinyon-juniper forest impressed travelers who encountered it, even in the southern desert regions.



## 2. Mountain Range Observations

Numerous records of singleleaf pinyon distribution have been reported at the mountain range level. Stretch (1867, 1869) characterized the Shoshone Mountains as having an abundance of wood, and the White Pine Range as “all quite densely covered with the usual growth of timber”. He described the Toiyabe Range as originally having been in many places “covered with the nut pine and juniper”, while wood was scarce in the Fish Creek Range. The Diamond Mountains were reported to have “a thrifty growth” of nut pine and mountain-mahogany. Of the Worthington Mountains he reported in 1867 the “whole range is well timbered with nut pine”, but in 1869 mentioned only “a small supply of timber”, the difference possibly due to heavy cutting in the interim.

Raymond (1869) described the high ridge of Mt. Irish, about five miles long and one-half to two miles wide, as well covered with nut pine and cedar.

According to White (1871) there was wood in abundance in the Schell Creek Range which featured such well-wooded mining districts as the Piermont, Nevada, McDugal, Patterson, Cooper and Fairview; as well as the Antelope Range, the Pine Grove Hills, the Snake Range and the Egan and Cortez Mountains. The Snake Range was home to the Snake, Sacramento, Pleasant Valley, Clifton, Lincoln and Shoshone Mining Districts, all of which were well supplied with pinyon. The low hills of the Ruby Mountains were covered with nut pine, juniper, and mountain-mahogany; and the hills surrounding Tem Piute Peak – the Timpahute Range – were covered with pinyon and juniper. The area 30 miles south of Clover Valley – apparently a reference to the Cherry Creek Range, perhaps including Spruce Mountain, was described by White (1871) as “rolling country principally covered by nut-pine and cedar”.

Wheeler (1872) found the Humboldt Range to be “pretty well covered by cedar and nut pine”, and reported that wood occurs in abundance in the “Candolara” (Candelaria) Hills. Wheeler also reported of the Silver Peak Range in Esmeralda County that its timber extended twelve to fifteen miles along the summit of the range, in a belt eight to ten miles wide, consisting of singleleaf pinyon, Utah juniper, and

mountain-mahogany, which "is small but good for that country and plenty of it".

Hough (1878) quoted Mrs. E. R. Chase of Wells, NV: "The range east of the Humboldt Range is covered on its upper surface with piñon pine, and its lower part with juniper. The former supplies all the country hereabout, and the towns along the railroad, with fuel, and it is nearly all the timber in the eastern portion of Nevada. It is rapidly disappearing under the demands of the neighboring towns." Mrs. Chase was apparently referring to the Wood Hills, possibly the Pequop Mountains. The Humboldt Range referred to is now the East Humboldt Range.

Recent investigation by Ko et al. (2011) of "land-use legacies", i.e. forensic evidence of past harvesting such as stumps and charcoal oven platforms, have shown that the Simpson Park Range was the site of logging of pinyon and juniper during the mining era, though we find no mention of that in our historical references.

After transiting southern Nevada, Coville (1893) reported of singleleaf pinyon that "All along the eastern slope of the Sierra Nevada, as far northward as the expedition went, and southward to the mountains about Antelope Valley, as well as in all the higher peaks eastward to the Colorado River, the tree occurred abundantly". The Nevada desert ranges upon which it was observed by the Biological Survey of the Death Valley were the White, Grapevine, Charleston (Spring), Magruder, Pahroc, Gold, and Virgin Mountains.

Merriam (1893) traversed the same areas as Coville. He reported singleleaf pinyon to be common in Nevada in the Charleston Mountains where nut pine and cedar "abounds" in frequent localities for 50 miles, in the Pahroc Mountains, and on Gold Mountain and Mt. Magruder. Of the latter range he reported "Mount Magruder is notable for the luxuriance of the nut pine forests which clothe its higher hills and peaks, and has long been a favorite resort of the Paiute Indians, who speak of it as 'Nut Pine Mountain'....The trees often attain a height of 12 or even 15 meters (40 to 50 feet) and a diameter of half a meter (nearly 20 inches)."

The Pine Nut Range and Washoe Mountains (Virginia Range) were also reported by several observers to have been heavily wooded with pinyon-juniper forest. They will be discussed below in connection with their deforestation. Several additional ranges have been named by Carlson (1974) as the locations of Mining Districts reported in the 19<sup>th</sup> century to have harbored pinyon-juniper forests. They are the Bristol, Grant, Groom, Highland, Mountain Boy, Kinsley, Palmetto, Reveille, and Sulphur Springs Ranges and Peavine Mountain.

The mountain ranges described in the reports cited above are widely scattered across virtually all sections of the state that are within the distribution area of singleleaf pinyon (Fig. 2).

### 3. Contemporary Mining District Reports

Mining Districts in Nevada were areas designated by name, with defined but varying boundaries containing one to many mines. For example, in the first two quarters of 1870 there were at least 89 mines active in the White Pine District, and many inactive at the time the report was written (Raymond 1873). Districts were organized by the miners for governance in areas outside the sway of state or county law. Stretch (1867) listed 114 districts and the number swelled to 182 by the early 20<sup>th</sup> century (Tingley 1998). Nevada State Mineralogists, county assessors, and other officials compiled mining statistics largely on a district basis.

Examination of the references consulted in this study disclosed 78 districts about which comments were made on the availability of wood for use as fuel, either as cordwood or charcoal, for industrial or domestic use. Many of the districts produced refractory silver ores that required smelting or roasting, and the railroad infrastructure to deliver coal from faraway coal-fields only began to be implemented in the third decade of mining (Charlet 2008). Therefore, wood resources were critically important and were consumed in immense quantities. It was advantageous to have accessible fuel wood in close proximity to the mines and mills, and the most important wood was that of singleleaf pinyon, which made superior charcoal (Lanner 1981). Thus it was necessary to appraise the wood resources in the districts as an indicator of economic viability.



Of those 78 districts, 20 (26%) were listed in more than one reference, allowing us to evaluate the consistency of the reporting. For example, the wood resources of the Freiberg (or Freyberg) District were described by White (1871) as "nut pine sufficient for mining purposes" and by Wheeler (1872) as "Timber sufficient for fuel and building". Of the Shoshone District in the Snake Range, Stretch (1867) reported "fuel abundant", Raymond (1870) "well wooded", and White (1871) "nearly the whole space described is covered with nut pine". Multiple reports of the same district were generally similar in 18 (90%) of the 20 districts with such reports.

Districts described as having "limited", "a small quantity", or an absence of pinyon resources were eight in number (10%). At least four of these were on the Humboldt River (Battle Mountain) or well north of it (Independencia, Pueblo, Vicksburg) and outside the range of singleleaf pinyon.

Those in which the terms "densely wooded", "abundant", "plenty", "good supply", or "fine supply" appeared, numbered 40.

Districts in which nut pine was said to be "inexhaustible", or the area "covered" or "well-wooded" numbered 16; and districts that had a "sufficiency" or "supply" of nut pine numbered 18. Several districts were described with more than one of these adjectives. The terms used in characterizing the pinyon resource of 32 Mining Districts by Stretch in 1867 and 1869 appear in Table 1.

Pinyon pine-bearing districts were scattered from the Snake Range on the Utah boundary in the east, to the Pine Nut Mountains facing California across the Carson Valley in the west; and from the Cortez Mountains just south of the Humboldt River in the north, to the Charleston Mountains a few miles from Las Vegas in the south. It is apparent that the majority of Mining Districts, which were distributed mainly in the mountains, benefitted from their proximity to a widely spread forest that contained significant volumes of cordwood.



## **Elevational Distribution**

### **1. The Lower Forest Border**

Here we contrast the elevation of the lower border of the pinyon-juniper forest as it was reported in the 19<sup>th</sup> century and by Wilson (1941), with its present-day elevation. Most reports of rapid pinyon-juniper forest spread concern expansion at the lower forest border into sagebrush steppe (Miller et al. 2008, Weisberg et al. 2007). Such spreading might occur down alluvial fans, or more generally down the gentle lower slopes or bajadas. Only a few germane 19<sup>th</sup> century observations could be found.

According to Muir (1918) the lower forest border was at the base of the mountains. Base and forest border elevations at two locations each, on the east and west slopes of three ranges that Muir climbed – the Shoshone, Toiyabe, and Toquima Ranges – are shown in Table 2.

Muir's statement that the forest border coincides with the base of the mountains holds up well for the Shoshone Mountains and the Toiyabe Range, though less precisely for the Toquima Range. However, there is no evidence of the forest border at these locations having expanded into the valleys. The placement of forest borders on the DeLorme (2008) maps reflects their elevation at a point in time, and land-use or natural events may have influenced those locations before or since. For example, trees may have invaded lower on the slopes, but were removed by chaining prior to the DeLorme mapping.

Coville (1893) reported that singleleaf pinyon grew from 5,100 ft. elevation on the west slope of the Charleston Mountains. We found the mean of lower forest border elevations on this slope near the mouths of Carpenter and Wallace Canyons and below Mount Stirling to be 5,867 ft. Coville also reported the lower pinyon-juniper forest border on the south slope of Gold Mountain to be 6,800 ft. Our analysis of the small forest area of this minor mountain finds 7,000 ft. as more typical. These observations do not indicate a downslope expansion of the forest edge.

According to Wilson (1941) the pinyon-juniper belt in the Pine Nut Mountains adjoins the sagebrush steppe on the east side at about 5,500 ft. At three locations on that slope, however, mapped elevations of the lower border have a mean of 6,000 ft. (Red and Mill Canyons and Rice Peak). Again, there is no evidence of lowering of the forest border in the approximately 72 years elapsing between observations. This is consistent with the earlier information cited above.

## 2. The Upper Forest Border

Pinyon-juniper forest expansion up mountain slopes has been suggested (West 1988, Weisberg et al. 2007)). On mountains higher than the historic upper limit of pinyon-juniper forest this might require replacement of ponderosa pine (*Pinus ponderosa* Douglas ex Lawson & C. Lawson) forest, limber pine (*P. flexilis* James) woodland, or mountain-mahogany woodland, depending on the location. Historic sources and current maps are of limited value in identifying such replacements.

Browne and Taylor (1867) reported pinyon-juniper forest attaining 9,000 ft, but without specifying any locations.

John Muir (Muir 1918) generalized an upper elevation of 8,000 to 9,000 ft., with the result that, "viewed comprehensively" the forest sweeps on "complacently over ridge and valley, and lofty crowning dome". In three of the ranges that Muir climbed, as depicted on the DeLorme topographical maps (DeLorme 2008), woodland shading is continuous from summit ridges to the mountain base. These are the Shoshone Mountains from Buffalo Peak (9,036 ft.), South Shoshone Peak (10,052 ft.) and North Shoshone Peak (10,313 ft.); the Toiyabe Range from Mahogany Mountain (10,970 ft.), French Peak (10,779 ft.) and Toiyabe Range Peak (10,960 ft.); and the Toquima Range from Little Table Mountain (9,756 ft.) and Mt. Wilson (9,205 ft.). It is not possible however to determine from the maps how much of that shading represents pinyon-juniper forest, and how much might indicate subalpine woodlands of limber pine.

Wilson (1941) described the pinyon-juniper forest of the Pine Nut Mountains and the Washoe Mountains as climbing "over the upper slopes to dominate the landscape".

According to Charlet (personal communication, July 2011), the highest actual record of singleleaf pinyon in Nevada is from 9,990 ft. on Hayford Peak in the Sheep Range; and the uppermost known limit of the pinyon-dominated woodland is 8,766 ft. in the Snake Range.

## PINYON-JUNIPER FOREST RECOVERY FROM DEFORESTATION

Deforestation proceeded rapidly after mining began in Nevada. Sargent (1879), for example, cited "the terrible destruction of forest, which follows, both on public and private domain, every new discovery of the precious metals"; and added, in 1880, that the pinyon "...will soon be exterminated, largely made into charcoal (cited in Strachan 2011)". Adding to the devastating effects of ordinary deforestation was the reported practice of pulling up roots, stumps and brush from cutover areas (Young and Budy 1979).

The extent of deforestation can only be reconstructed from fragmentary information on such imprecise parameters as amount of charcoal used in smelting, bushels of charcoal per cord of wood, cords of wood per acre; cords of wood used for home heating and cooking over the decades, and for generating steam in mining machinery; acreage cleared for home sites, mill-sites, transportation corridors, pasturage, agricultural needs, structural needs, posts for fences, corrals; number of railroad ties, cordwood used to fuel locomotives, and many other factors. Lanner (1981) very roughly estimated about 750, 000 acres were denuded to fill these needs. Young and Budy (1979) estimated that by 1878, 600,000 acres had been denuded within 35 miles of Eureka.. Charlet (2008) estimates that in 1874-1879, 1.14 million cords of fuel wood were consumed in Virginia City, the output of about 80,000 acres. He estimates that over 33 years Nevada's railroads consumed the output of 63,300 acres. Charlet (2008) concludes that "...while the pinyon-juniper forests were not wiped out, their range was significantly decreased...." He does not estimate a total acreage of deforestation. A comprehensive estimate remains elusive.

Below are some especially well-documented episodes of deforestation:

### **Eureka Area**

According to Earl (1979) by 1878 the pinyon-juniper forest around Eureka in central Nevada had been denuded to a distance of 50 miles. A circle of this magnitude includes the Diamond, Butte, and Roberts Mountains; and the White Pine, Antelope, Pancake, Monitor, Mountain Boy, Fish Creek, Simpson Park, Sulphur Springs, and Maverick Springs Ranges. We found historical reports only for the Diamond Mountains and the White Pine Range (Stretch 1867, 1869), both of which were noted to be wooded. In addition are the recent reports by Reno (1994) on the Roberts Mountains, and by Ko et al. (2011) on the Simpson Park Range mentioned earlier.

Present-day forest cover is indicated by woodland shading for all twelve of those ranges on the DeLorme maps, from the base of the ranges well up the slopes. In addition, Charlet (1996) reports singleleaf pinyon to be "present" in the Antelope, Pancake, and Maverick Springs Ranges, and to be "abundant" in pinyon-juniper woodlands in all the others. Therefore all twelve mountain ranges within the reported potential area of deforestation around Eureka are now to some degree forested.

A dramatic report of deforestation in the Eureka area can be found in a letter of April 28, 1887 to the Secretary of the Interior and the Commissioner of the General Land Office in Washington, D. C., from Thomas Haydon, the United States District Attorney for Nevada (De Knight 1889). Haydon wished to prosecute cases of timber trespass on public land. In making his argument, he pointed out that for years hundreds, maybe thousands, of woodcutters had been "systematically engaged in cutting off into cordwood or burning into charcoal thousands of acres of timber on land belonging to the Government....In the region about Eureka ...there has probably been several hundred square miles of land covered with a growth of nut-pine timber from 8 to 10 inches in diameter to 30, and from 8 or 10 feet to 30 feet in height, and with cedar considerably less in diameter and height (that) have been swept bare, and probably one or two million cords of wood have



thus been taken off of public land....” Of wood hauled by the Eureka and Palisade Narrow Gauge Railroad, Haydon writes “...four-fifths of it is not over 5 inches in diameter, and scarce one tree out of fifty is over 8 inches in diameter. The fact is, all that land was culled and cut over once, taking all trees of any size, and now they are cutting it over a second time and sweeping every young tree and bush over 2 or 3 inches in diameter.” Haydon found similar conditions around Austin, White Pine, Belmont, Pioche, and “...every other large mining camp in this State....” Haydon’s Eureka region information was verified by a mine superintendent and a marshal.

Haydon’s unique contribution is to document the tree sizes involved in the indiscriminate cutting of second-growth forest less than two decades after mining began; and to state that cutting practices were similar in all major mining districts. This indicates that deforestation was both widespread and sustained.

### **Cortez Mining District**

The Cortez Mining District was established in 1863 in the Cortez Mountains of north-central Nevada. Stretch (1867) characterized Mt. Tenabo, which dominates the area, as being “covered from base to thousands of feet up its side to the vein” with nut pine. About 1868 the mill updated its ore processing methods, now requiring large amounts of charcoal made from singleleaf pinyon. In his report of 1869 Stretch again referred to a “whole mountain covered with nut pine”. An archaeological study made by Hattori and Thompson (1986) has utilized repeat photography and tree-ring dating of trees and stumps to synthesize a history of deforestation and recovery at this location. Their data are used here.

The original forest at Cortez extended from the bajada at about 5,770 ft. elevation to about 8,750 ft. atop Mt. Tenabo (similar to Muir’s [1918] generalization). Mining and ore processing continued with some breaks until 1928. During much of that period the effects of clearcutting to fill the needs for charcoal, cordwood, and construction timber had a dramatic impact on the landscape, though some scattered mature trees survived the intensive cutting, apparently retained for reasons now unknown.

Matched photographs show a slope of Mt. Tenabo that was believed intensively logged between 1886 and 1892, taken about 1900; and again in 1983. The older photo shows an open savanna-like aspect of scattered lines of small bushy trees giving perhaps 10% ground cover. The more recent photo shows an almost 100% ground cover of dense pinyon-juniper forest. Hattori and Thompson speculate that, judging from the age of a small number of stumps on the bajada, a post-1840 expansion of the forest may have occurred, but they offer limited data.

According to Charlet (1996), singleleaf pinyon is found today in pinyon-juniper woodland in the extreme southern tip of the Cortez Mountains. The DeLorme map shows woodland shading throughout the Cortez and Mt. Tenabo area, and on a series of un-named hills extending to the southwest. The data and photographs presented by Hattori and Thompson (1986) clearly establish that the forest at Cortez described by Stretch in 1867 and 1869 was severely deforested, yet has returned to dominance.

### **The Pine Nut Mountains and Washoe Mountains (Virginia Range)**

The Pine Nut Mountains, which form the eastern wall of Carson Valley in extreme western Nevada, were the source of huge volumes of singleleaf pinyon cordwood and charcoal from the opening of the Comstock Lode in 1859, continually, to well into the 20<sup>th</sup> century (Wilson 1941). As early as 1867, just eight years after mining on the lode began, Stretch (1867) reported that while the Pine Nut Mountains had formerly yielded a large supply of fuel, the hills were now "largely bare". The industrialized complex of Virginia City, Silver City, Dayton, Gold Hill and other towns then turned to the great conifer forests of the Sierra Nevada, while mining and smelting in the interior Mining Districts continued to depend on local resources of singleleaf pinyon and Utah juniper for their needs.

Stretch (1867) reported a very similar situation in the Washoe Mountains, a continuation of the Pine Nut Mountains north of the Carson River. This range too was covered with pinyon-juniper forest when the Comstock Lode was discovered, but by 1867 "... they have

been extirpated, and Virginia depends for its supply of wood and timber chiefly on the slope of the Sierra Nevada....”

Wilson (1941) reported on field work conducted in 1936-1939, by the Forest Survey of California and Western Nevada, in what he explicitly referred to as second-growth forest that had replaced the virgin pinyon-juniper forest. This second-growth had been exploited as it came back. The forest covered nearly 40 percent of Douglas County and continued over the upper slopes where it dominated the landscape of the Pine Nut Mountains. Those stands mostly ranged from 20 to 60 years of age, with the pinyon trees commonly 4-10 inches in diameter and 8-20 feet tall, and were yielding appreciable income from cordwood and pine nuts. Wilson (1941) inventoried more than 138,000 acres of second-growth stands in the Douglas County portion of the Pine Nut Mountains, and almost 30,000 acres in the Ormsby County (now Carson City County) portion.

According to Charlet (1996) singleleaf pinyon is dominant in extensive pinyon-juniper forests throughout the range, extending nearly to the highest summits. Woodland shading is shown throughout the range (DeLorme 2008).

These examples of pinyon-juniper forest recovery from deforestation demonstrate the reproductive vigor and resilience of this native forest type.

## DISCUSSION AND CONCLUSIONS

Contemporary reports of Nevada's 19<sup>th</sup> century pinyon-juniper forest do not support the concept of a sparse woodland restricted to refractory sites and forming open savannas.

On the contrary, the archives we consulted depict it as widespread, continuous and dense. By widespread, we cite locations of many forested mining districts. By continuous, we refer to many reports of forests spanning mountain ranges. And by dense, we point to repeated comments on the availability of fuel wood to support a major industry (Table 1); and the absence of comments on scattered-tree savannas. The observers – explorers, a naturalist, mining officials,

scientists, a prosecuting attorney – were men of serious purpose. Their reports had financial significance. And all had eastern U. S. or European roots that familiarized them with dense forests.

By 1878 Muir was a seasoned observer of Sierra Nevada trees, forests, and land forms. He depicted the north-south oriented mountain ranges of central Nevada as densely covered over broad areas with pinyon-juniper forests. Despite the progression of deforestation Muir saw no imminent threat to so vast a resource. Muir's description of the ground beneath the forest is curiously similar to present-day conditions beneath forest said to be invaded shrubland.

The less detailed and specific observations of Browne and Taylor (1867) and of Wheeler (1872) do not contradict Muir's observations.

The lower borders of the forest on some of the mountains Muir climbed, appear not to have invaded valleys below. Nor have those of the southern mountains reported on by Merriam (1893) and Coville (1893).

Mining was conducted overwhelmingly in the mountains, where ore bodies are near the surface. It is unlikely that need and availability would be so frequently matched if the forest was limited in area and savanna-like in structure. Nor did any observer mention mines or mills running short of fuel.

The recovery from deforestation in the mining era, and chaining in the past few decades, suggests that efforts to remove these forests from the landscape (Miller et al. 2008) will be futile. Range managers eradicating forest growth now rely on the "bullhog", a machine that grinds the trees it topples into mulch, to eliminate the problem of small surviving trees (Charlet 2008). But this overlooks the role of animal dispersers, especially the pinyon jays and Clark's nutcrackers that can be relied on to cache pinyon seeds across gaps in the fragmented pinyon-juniper forest (Chambers et al. 1999). These birds brought singleleaf pinyon into the Great Basin several thousand years ago (Lanner 1983) and there is no reason to think they will be incapable of keeping it there. In addition to the long-distance dispersal



effected by these members of the family Corvidae, local dispersal of pinyon seeds by rodents will probably contribute to the filling in of existing stands (Chambers et al. 1999, Vander Wall 1997). The case studies of forest recovery in deforested areas testify to the effectiveness of the pinyon and juniper dispersers.

The lesson of recovery from deforestation is that Nevada's pinyon-juniper forest is an adapted and resilient plant community that should be managed sustainably in order to gain the many known benefits of forests. These include production of wood products and pine nuts, habitat for countless native species of animals and plants of higher and lower forms, carbon dioxide sequestration, moderation of microclimates, the windbreak effect, and protection of the soil in a semi-arid climate. It would be biologically and economically wasteful to attempt the deforestation of these areas in order to restore them to a condition "logically inferred", but not scientifically demonstrated, to have existed in the 19<sup>th</sup> century.

### ACKNOWLEDGEMENTS

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Table 1.Characterization of the singleleaf pinyon (*Pinus monophylla*) resource at Nevada Mining Districts as described by the Nevada State Mineralogist (Stretch 1867, 1869).

Pinyon resource	Mining District
Scarce, hardly any	American, San Antonio
Limited amount, small quantity	Battle Mountain, Echo
Abundant, good supply, fine supply	Blind Springs, Buckeye, Esmeralda, Eureka, Great Basin, Highland, Montgomery, North Twin River, Osceola, Pahrnagat, Peavine, Red Mountain, Reveille, Roberts, Santa Rosa, Shoshone, Union
Well wooded or timbered, dense or thick growth, large quantities, large areas covered	Cortez, Palmyra, Robinson, Springfield, The Jackson, Wilson's, Worthington
Inexhaustible	Mammoth

Table 2.Mean elevations at the mountain base and lower forest border at four locations of three ranges said by Muir (1918) to have pinyon-juniper forest belts descending to the mountain base. Mountain base defined as upper limit of bajada or pediment.

Range	Locations	Mean base elevation, feet	Mean forest border elev.
Shoshone	Cole, Mitchell, Mission, Buffalo Canyons	7,000	6,950
Toiyabe	Crooked, New York, Dry Canyons; Last Chance Creek	6,450	6,525
Toquima	Sam's, Mill, Willow Canyons; Manhattan Gulch	6,600	7,100

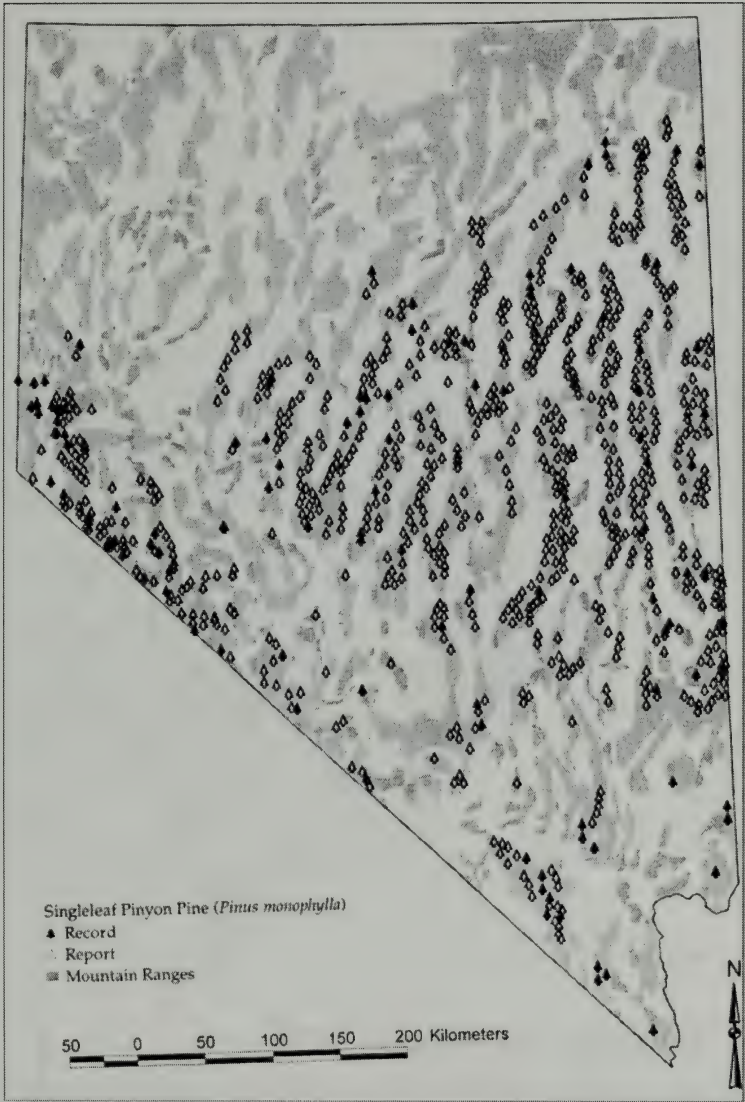


Figure 1. Present-day distribution of singleleaf pinyon in Nevada. From Charlet (1996) by permission (see Acknowledgements).

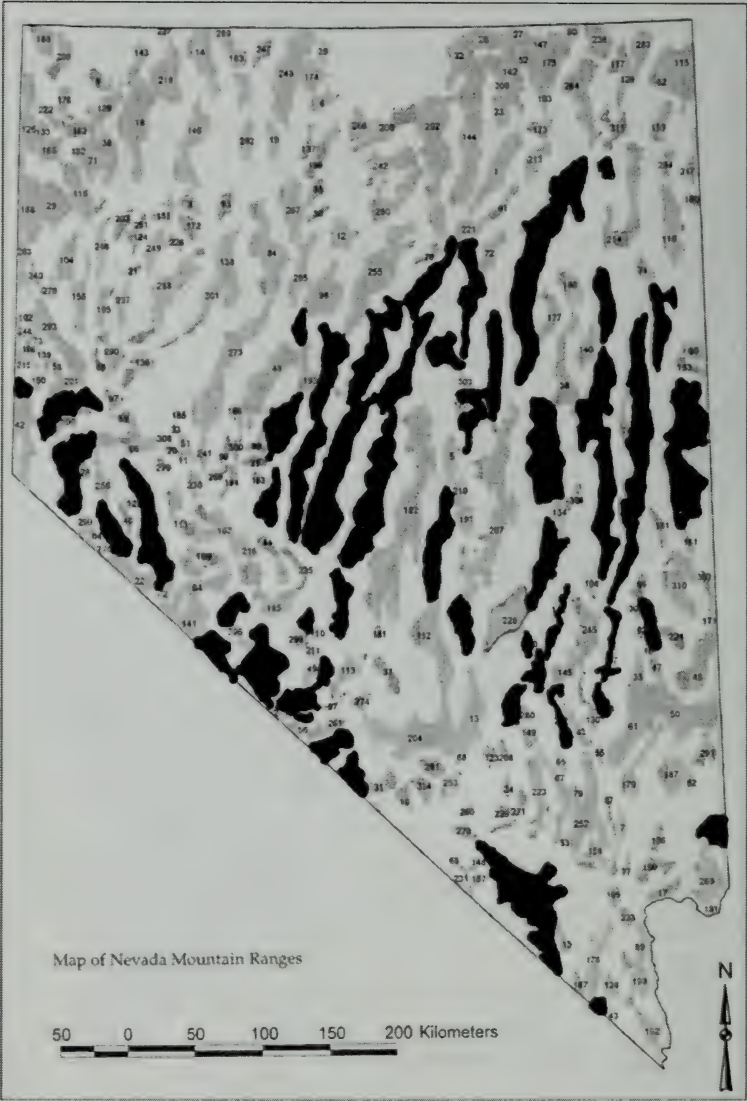


Figure 2. Nevada mountain ranges said to have major cover of pinyon pine in the 19<sup>th</sup> century. See text for individual reports. From Charlet (1996) by permission (see Acknowledgements).





Figure 3. Singleleaf pinyon-Utah juniper forest near Austin, Nevada.

**PHYLOGENETIC RELATIONSHIPS AMONG GENERA OF  
THE SUBTRIBE ONCIDIINAE (EPIDENDROIDEAE:  
ORCHIDACEAE) AND A NEW GENUS: *SANTANDERELLA***

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**ABSTRACT**

*Santanderella*, a new genus of orchids from Colombia with the type species, *Santanderella amado-rinconiana*, related to *Macroclinium* and *Notylia*, is analyzed both at the phenotypic and genotypic levels. Phylogenetic trees related to genomic *matK-trnK* and *ITS1-5.8S-ITS2* sequences are presented to support the proposal of a new genus. *Phytologia* 93(3):388-406 (December 1, 2011).

**KEY WORDS:** Orchidaceae, Oncidiinae, *Santanderella*, Colombia, *matK-trnK*, *ITS1-5.8S-ITS2*.

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An orchid plant belonging to the subtribe Oncidiinae (sensu R. Dressler, 1981) and showing affinity with the genera *Notylia* Lindl. and *Macroclinium* Barb. Rodr., was collected by Jonathan Amado in Floridablanca, Santander, Colombia, and reported by Orlando Rincón in 2009 (Figure 1A).

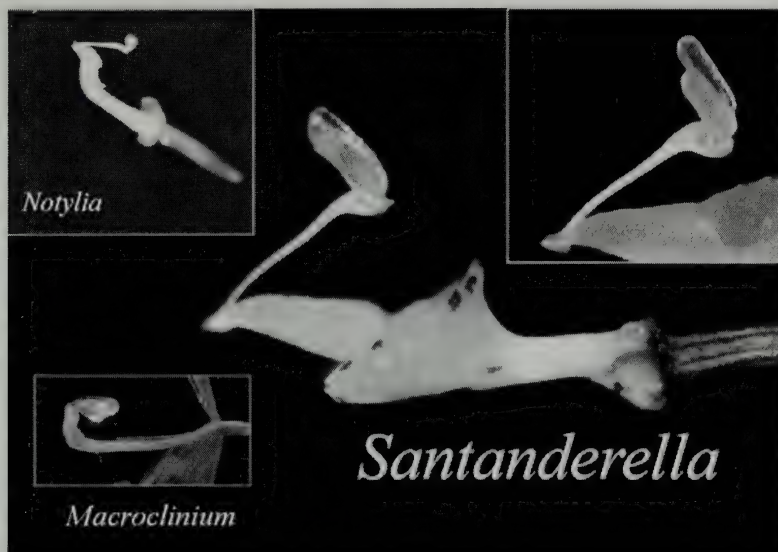


Figure 1A. *Santanderella amado-rinconiana* P. Ortiz. Comparison of the columns of the three related genera: *Notylia*, *Macroclinium* and *Santanderella*. Notice the peculiar shape of the column and the pollinia of *Santanderella*.

A number of characters of this specimen showed affinity with species of *Notylia*: the epiphytic, caespitose plant with unifoliate pseudobulbs and conduplicate leaves, the many-flowered racemose inflorescence, the rather large dorsal anther, the two pollinia with a thin and elongated stipe, and the ventral stigma as a narrow, longitudinal slit. Many of these characters are also found in the genus *Macroclinium*. But at the same time, the structure of the column and the pollinia, in addition to the characters of the sepals and petals, and especially of the lip, presented marked differences when compared to those of the close genera.

The plant we are dealing with has flowers that do not open fully (which seems to be a general condition of all the plants of this species seen by the collectors), with narrow sepals and petals, and a lip

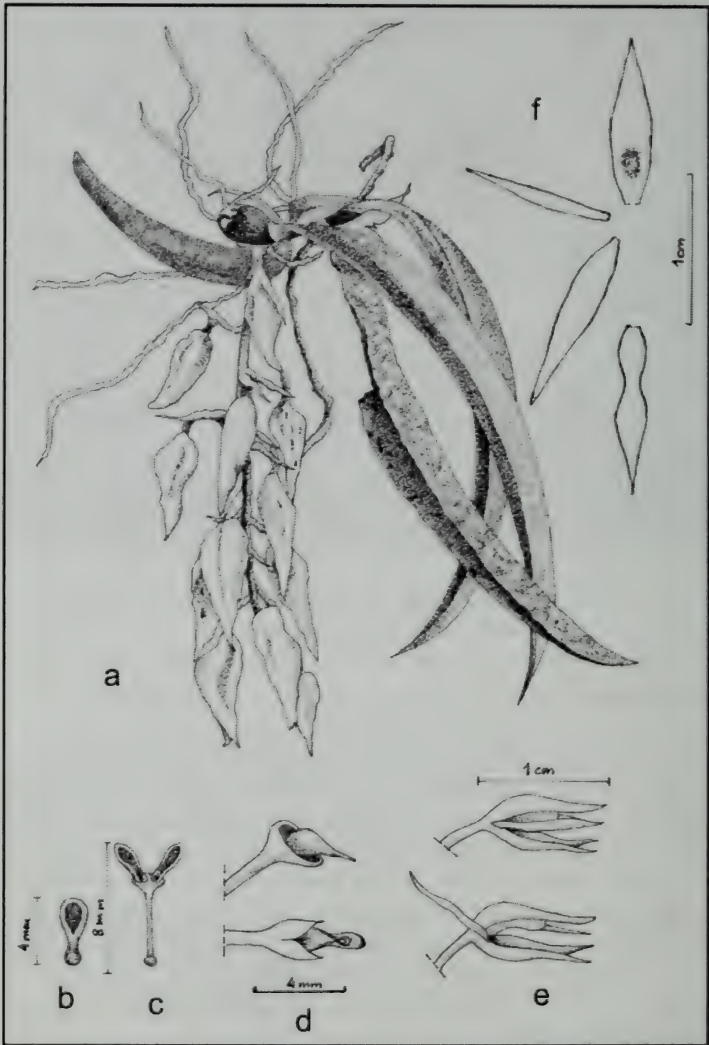


Figure 1B. *Santanderella amado-rinconiana*: **a**- Plant; **b**- Anther; **c**- two elongated, laminar, concave and yellow pollinia, affixed to a stipe with a triangular apex, then thin, 4 mm long; **d**- column, side and ventral views; **e**- Flower; **f**- Sepals, petals and lip.



that is different from all the “notyliiform” lips so far seen. It is very narrow, with a pair of small rounded lobules at the base, then turns narrow again, and then widens a little, with a subacute apex. There is no callus. It reminds the flowers of *Macroclinium*. The column is relatively short, with a basal part terete, and it widens apically into two obtuse, irregular wings, which ventrally merge together forming an acute angle. There is a clinandrium with rather high walls and inside the cavity the rostellum stands out, which is thick and high at the base and extends forward into a sharp point. The column does not bend backwards as in most *Notylia* species, but is rather straight. On the ventral part of the rostellum the stigma can be seen as a narrow slit. The anther is similar to those of *Notylia* and *Macroclinium*. But the pollinia are most remarkable. There are two pollinia, as in all of the Oncidiinae, but unlike the pollinia of *Notylia* and *Macroclinium*, they are quite large and elongated, flattened and concave. This type of pollinia, as far as we know, is not found in any species of *Notylia* or *Macroclinium*. The Oncidiinae genera close to *Notylia* have been defined and characterized in different ways, as can be seen in the study published by Pupulin (1997), to which we refer for further information. According to his study, the main difference between *Notylia* and *Macroclinium* lies in the shape of the leaves: dorso-ventrally flattened (*Notylia*) vs. laterally flattened (*Macroclinium*). The leaves of *Santanderella* are dorso-ventrally flattened, but are V-shaped.

We came to the conclusion that a new genus had to be established to accomodate this new species and so, on the basis of the phenotypic analysis, it was published in *Orquideología* (Medellín) 27(2): 167-178, 2011 (sub 2010) (Ortiz, 2011). Although establishing monotypic genera is not ideal, we cannot stretch out the limits of the genera to force incongruous elements into established genera. On the other side, this is not the only monotypic genus within this group (equally monotypic are *Notyliopsis*, *Sarmenticola*, *Chelyorchis*, *Hintonella*, *Hofmeisterella*, and *Schunkea*).

We then proceeded to a molecular analysis to determine the phylogenetic affinities of this eventual new genus with different orchids, which have already been reported by us and others in GenBank including: *Santanderella amado-rinconiana*, *Macroclinium xiphophorum*, *Notylia incurva*, *Notyliopsis beatricis*, *Oncidium*

(*Trichocentrum*) *lanceanum*, *Oncidium ornithorhynchum*, *Oncidium cultratum*, *Oncidium* (*Otoglossum*) *globuliferum*, *Oncidium fuscum*, *Brassia* sp., *Macradenia brassavolae*, *Trichocentrum pulchrum*, *Oliveriana ortizii*, *Telipogon nervosus*, *Oncidium* (*Trichocentrum*) *carthagenense*.<sup>1</sup>

In the present study, we present the phylogeny of the new genus *Santanderella amado-rinconiana* using plastid and nuclear markers (*matK-trnK* and *ITS1-5.8S-ITS2* sequences) and evaluate the classification systems previously proposed by Ortiz (2011), based on morphological characters.

## MATERIALS AND METHODS

### Taxon sampling

We first sampled 15 currently recognized species of the subtribe *Oncidiinae* (Pridgeon, 2009) available on local crops that were not previously reported in GenBank and performed phylogenetic analysis comparing these genera with *Santanderella amado-rinconiana* (Table 1). We only included *matK-trnK* and *ITS1-5.8S-ITS2* sequences of the closest taxa, according to the most recent classification of the family (Chase et al, 2005), as can be seen on Table 2. The comparing genera thus included the following: *Macroclinium*, *Notylia* and *Macradenia*. *Notyliopsis* was selected as an outgroup, following the principles stated by Felsenstein (1985) and Swofford (2002).

### DNA extraction

Plant tissues were dried using silica gel and stored at 70°C (Chase and Hills, 1991). DNA was extracted using a modified CTAB protocol (Doyle and Doyle, 1987). Approximately 0.25 g of green tissue was ground under a mortar and was transferred to a 1.5ml eppendorf tube. Seven hundred microliters (µl) of hot (65°C) CTAB buffer (0.02 M EDTA, 1.4M NaCl, 0.1 M Tris pH 8.0, 2% CTAB, 0.7%

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<sup>1</sup> For an alternative nomenclature used recently by other authors (included here in parenthesis), refer to the Kew webpage "World Checklist of Selected Plant Families", in: [apps.kew.org/wcsp/home.do](http://apps.kew.org/wcsp/home.do)

v/v DTT, 2% soluble PVP) was added. The slurry was incubated at 65°C for 30 min with occasional shaking, followed by extraction with an equal volume of chloroform-isoamyl alcohol (24:1). Phases were separated by centrifugation for 10 min at 16,000g. The aqueous phase was removed and reextracted with chloroform-isoamyl alcohol. The aqueous phase was removed again and two hundred ninety one  $\mu$ l of isopropanol and forty  $\mu$ l of ammonium acetate 7.5 M were added, gently mixed, and incubated at -20°C overnight. The DNA was pelleted at 20,000g for 5min. The pellet was washed briefly in 76% ethanol/0.01 M sodium acetate and then centrifuged for 5 min. The supernatant was removed; the pellet was air-dried and resuspended in 100  $\mu$ l of TE Buffer (10m MTris, pH 8, 0.1 mM EDTA).

### DNA amplification

When necessary, DNA was cleaned using a Pure Link PCR® purification kit (Invitrogen, USA) according to manufacturer's instructions. A 1482 bp fragment from the 30' end of the *matK-trnK* gene was amplified using primers 19F and 556R (Table 3) in the PCR. Each PCR had a final volume of 100  $\mu$ l and contained 10–20 ng of genomic DNA, 200 $\mu$ M each dATP, dCTP, dTTP and dGTP, 2.5 mM MgCl<sub>2</sub>, 0.5  $\mu$ M forward (19F - 390F) and reverse (556R and 1326R) primers, 1.25 U Taq DNA polymerase GO (Promega, USA) and 5 X of buffer green of Taq DNA polymerase GO buffer (Promega, USA). Cycling conditions were: initial melting at 94 °C for 5 min; 39 cycles of 94°C for 1min, 48.6°C for 1min, 72°C for 2 min; final extension was set at 72 °C for 15 min.

The amplification of the nuclear internal transcribed spacer (*ITS*) region sequences (also defined as *ITS1-5.8S-ITS2*) on the following species was reported by ourselves on GeneBank: *Notylia incurva*, *Notyliopsis beatricis*, *Santanderella amado-rinconiana* and *Macroclinium xiphophorum*. Fifteen additional *ITS* sequences (7 *Macroclinium* sp. and 8 *Notylia* sp.) were included in our phylogenetic analysis. The amplification of the *ITS1-5.8S-ITS2* region was conducted in a polymerase chain reaction (PCR) with the primer sequences proposed by Sun (1994) (Table 4). The reagent PCR volume of 100 $\mu$ l reactions contained: 5x Go taq Promega Buffer, 10  $\mu$ l of bovine serum albumine (BSA), 25mM MgCl<sub>2</sub>, 10 mM of each primer, 2  $\mu$ l of Promega Go Taq (5U/ $\mu$ l), 10mM of dNTPs, 4  $\mu$ l of dimethyl sulfoxide



(DMSO), genomic DNA (20 ng/ $\mu$ l) and 58  $\mu$ l of water. The PCR protocol included: one first step of initial denaturation 5 minutes (95°C), 30 cycles of 1 min denaturation (94°C), 1 min annealing (54°C), and 2 min, 30 s elongation (72°C), with two additional seconds elongation per cycle and a final elongation step of 7 minutes (72°C).

### DNA sequencing

PCR products were purified using a QIAquick DNA Cleanup System<sup>®</sup> (Qiagen, Germany) and sequenced using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit<sup>®</sup> (Applied Biosystems, USA), following the recommendations of the manufacturer. The sequencing products were analyzed by an ABI 3100 Avant Sequencer<sup>®</sup> (Applied Biosystems, USA). The sequences were assembled in Sequencher 3.0 (Gene Codes, Ann Arbor, Michigan, USA) and aligned manually in MacClade v. 4.08 (Maddison & Maddison, 2005). Gaps were coded separately and excluded from the analyses. Regions with ambiguous alignments were also excluded.

### Phylogenetic analysis

Maximum parsimony (MP) and maximum likelihood (ML) analyses were performed in PAUP\*, version 4.0b10 (Swofford, 2002). MP and ML heuristic searches used 1,000 replicates of random taxon stepwise-addition (retaining 20 trees at each replicate), tree bisection reconnection (TBR) branch swapping, and equal weighting of all characters. For ML searches, the best-fit model of nucleotide substitution and model parameters were determined for *matK-trnK* and for *ITS* using ModelTest 3.04 (Posada & Crandall, 1998); F81+I+G and K81uf+I+G were respectively identified as the most appropriate models of evolution for each of these data sets. Support was assessed with non-parametric bootstrapping; heuristic searches with 1000 replicates for MP and 100 replicates for ML were conducted using the same parameters as described above. Clades with bootstrap support of 50–74% were considered weakly supported, 75–89%, moderately supported, and 90–100%, strongly supported.



## RESULTS

The data sets for *ITS* and *matK-trnK* sequences presented different levels of variation and contained varied amounts of indels, as can be seen on Table 5. Specific *matK-trnK* gene sequences were generated for the new genus *Santanderella amado-rinconiana*, and for *Macroclinium xiphophorum*, *Notylia incurva*, *Notyliopsis beatricis*, *Oncidium (Trichocentrum) lanceanum*, *Oncidium ornithorhynchum*, *Oncidium cultratum*, *Oncidium (Otoglossum) globuliferum*, *Oncidium fuscum*, *Brassia* sp., *Macradenia brassavolae*, *Trichocentrum pulchrum*, *Oliveriana ortizii*, *Telipogon nervosus*, *Oncidium (Trichocentrum) carthagenense*. Sequences are available in GenBank (accession numbers provided in Table 1). Data in the combined data set (*ITS* and *matK-trnK*) contained several small gaps (up to 20 bp in length) and an aligned matrix with 1611 characters. MP analysis for this marker resulted in 6478 trees of 749 steps with a CI of 0.52 and a RI of 0.73; overall, 17.9% of the sites included in the analyses were informative (Table 5).

*ITS* sequences were obtained for *Santanderella amado-rinconiana*, *Notyliopsis beatricis*, *Macroclinium xiphophorum* and *Notylia incurva*. The corresponding MP search resulted in 3,414 trees of 179 steps (CI=0.65; RI=0.75). The aligned matrix resulted in 558 characters of which 7.9% were parsimony informative (Table 5). The ML search led to a single tree with  $-\ln L = 1807.26573$ . The topologies obtained through the MP and ML analyses were congruent with respect to all strongly supported clades. The ILD ( $P=0.001$ ) and Templeton tests (rival tree *ITS*,  $p<0.0001$ ; rival tree plastid,  $p=0.34$ ) suggested that the *matK-trnK* data set is incongruent with *ITS*. Furthermore, several contradictory relationships were found between the *matK-trnK* and *ITS* topologies (data not shown). Hence, *ITS* data sets were analyzed in combination with *matK-trnK* data sets through MP and ML analyses. Phylogenetic relationships among species were consistent in both ML and MP phylograms (Figures 2 and 3).

In the first step, *matK-trnK* and *ITS* sequences were used to perform a broader analysis on representatives of all Orchidaceae to test the monophyly of Oncidiinae, and also to explore their position within

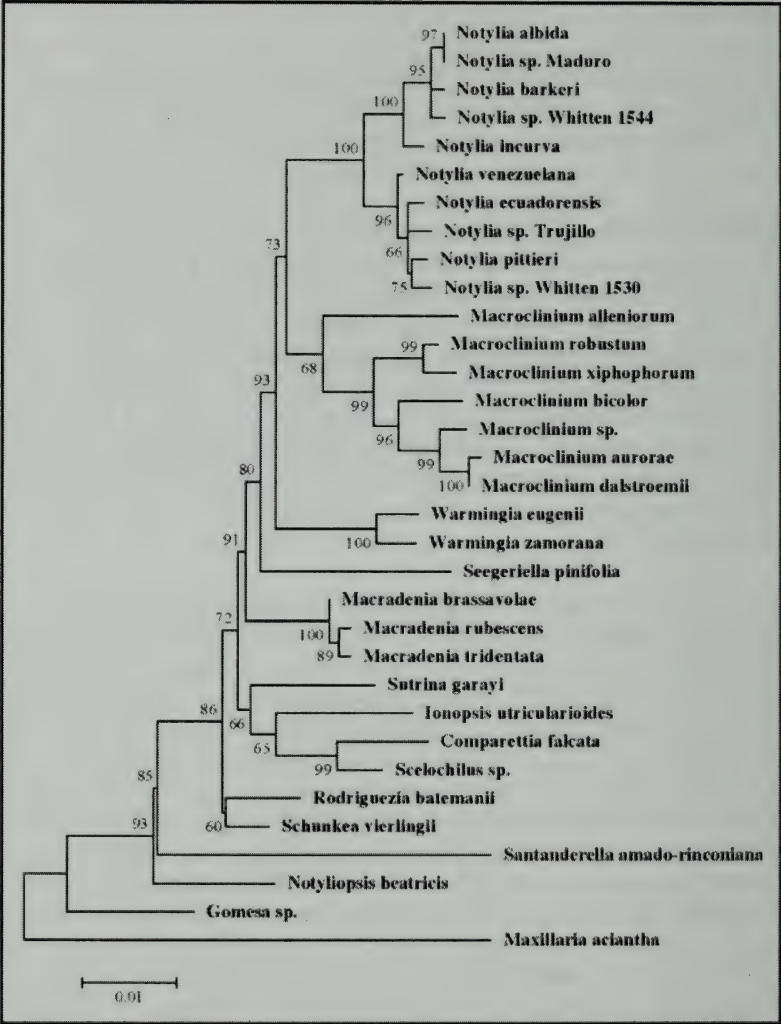


Figure 2. Maximum likelihood phylogram based on combined *matK-trnK* and *ITS* data.

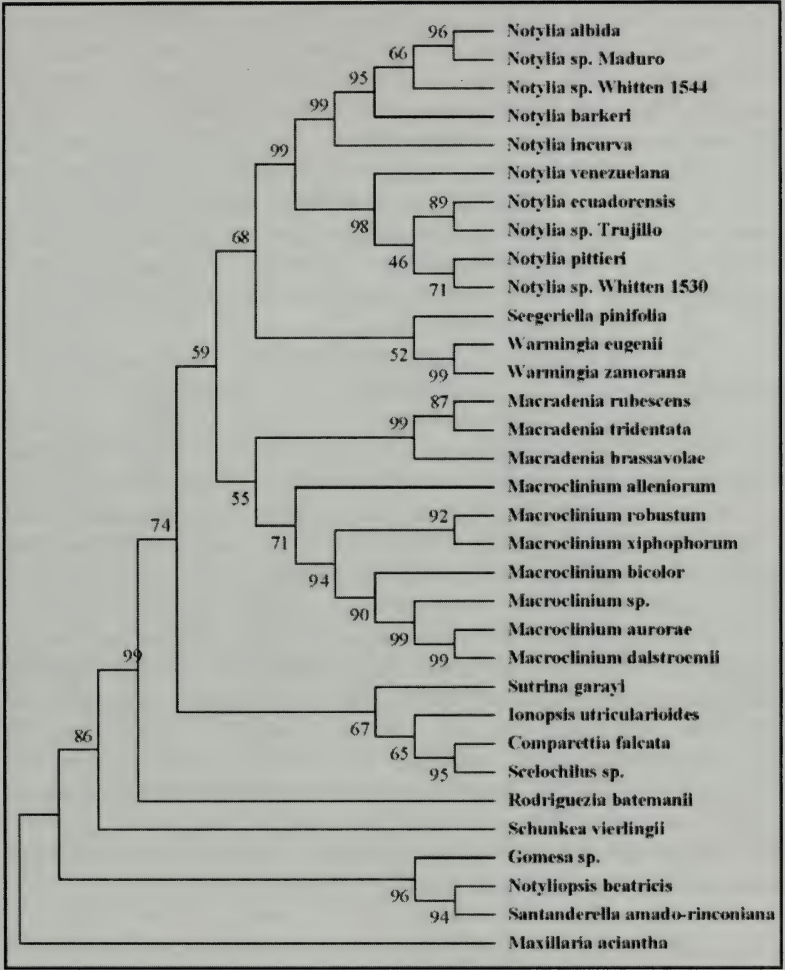


Figure 3. Maximum parsimony (MP) strict consensus topologies, combined analysis between *matK-trnK* and *ITS* sequences in the genera of the subtribe *Oncidiinae* close to *Santanderella*. Maximum parsimony bootstrap values are shown above branches.

the family performed on all the genera included in the phylogenetic three published by Chase (2005) (Table 1, Figures 2 and 3). Subsequently, more restricted analyses were performed in order to compare separately *Macroclinium*, *Notylia*, *Macradenia*, *Notyliopsis* and *Santanderella* based on their ITS 1-2 and *matK-trnK* sequences, then *Santanderella amado-rinconiana* and *Notylia* were compared on their ITS and *matK-trnK* sequences and, finally, *Santanderella amado-rinconiana* was compared to *Macroclinium* based on their ITS1-ITS2 and *matK-trnK* sequences (data not shown). Every phylogram confirmed the monophyly of the new genus *Santanderella amado-rinconiana*.

Restricted analyses of both *matK-trnK* and ITS sequences were performed in order to compare separately *Santanderella amado-rinconiana* with each taxonomic subgroup. When *matK-trnK* sequences were compared within the genus *Notylia*, we found that *Notyliopsis beatrix*, *Notylia venezuelana* and *Santanderella amado-rinconiana* appear as outgroups. In contrast, when *matK-trnK* sequences were compared within the genus *Macroclinium*, only *Santanderella amado-rinconiana* appears as an outgroup (data not shown).

When only *matK-trnK* sequences of *Santanderella* were compared within a wider sample population which included *Macroclinium*, *Notylia*, *Macradenia* and *Notyliopsis*, no clear-cut distinction was found between species belonging to those genera. However, four species, namely *Santanderella amado-rinconiana*, *Notyliopsis beatrix*, *Macradenia brassavolae* and *Notylia* sp. appeared to correspond to outgroups in this phylogeny. In the central clades a *Macradenia* species appeared to be closely related to *Macroclinium chasei* and *Macroclinium alleniorum* (data not shown).

Moreover, when ITS sequences from *Santanderella* were compared within *Macroclinium*, *Notylia*, *Macradenia* and *Notyliopsis*, both *Santanderella amado-rinconiana* and *Notyliopsis beatrix* appeared as outgroups. When nuclear genetic markers were compared, a clearer distinction was found between species belonging to the genera *Notylia* and *Macroclinium* which now appear clearly monophyletic (data not shown).



Furthermore, we incorporated additional analysis with a combined sequences (ITS and *matK-trnK*) in a pooled analysis with the most related genera that were included in the phylogenetic tree published by Chase (2005) based on *matK-trnK* sequences. We then selected *Maxilaria aciantha* as an outgroup, and we confirmed the particularity of two specific genera, namely *Santanderella amado-rinconiana* and *Notyliopsis beatrixis*, as compared to the other species of the subtribe *Oncidiinae* belonging to *Macroclinium*, *Macradenia* and *Notylia*. These two apparently monophyletic genera appeared on an outside cluster in relation to other monophyletic genera in this phylogeny both by the ML and MP approaches (Figures 2 and 3).

## DISCUSSION

In this study, we used one plastid molecular marker (*matK-trnK*) and a nuclear data set (*ITS*) to investigate phylogenetic relationships within the subtribe *Oncidiinae* and genera more closely associated to the new genus proposed as *Santanderella* (Ortiz, 2011). The *ITS1-5.8S-ITS2* markers produced congruent topologies while *matK-trnK* topologies suggested a slightly different scenario than the one recovered with the nuclear data. In the following paragraphs, we discuss the results from phylogenetic analyses, differences between the *ITS* and plastid topologies, and the implications of this results for the systematics of the new genus *Santanderella*.

Literature of molecular systematics of orchids is growing as can be seen in previously published reports (Pridgeon et al, 2001; Salazar et al, 2009) and also on GenBank databases, where 4710 sequences belonging to *Oncidiinae* have been reported on 793 species belonging to 73 genera, including 15 new species reported by ourselves. The results revealed that neither *Macroclinium*, *Macradenia*, *Notylia* and *Notyliopsis* show molecular phylogenetic affinity with *Santanderella amado-rinconiana*. However, as we consider that molecular phylogenetic affinity to determine a taxonomic category has to include phenotypic considerations, we combined phenotypic and genotypic criteria for the description and classification of this new genus.

The molecular approach confirms our first impression based on phenotypic characters, as the specimen proposed as a new genus (Ortiz, 2011) appears indeed isolated on a different branch both by *matK-trnK* and *ITS* maximum parsimony strict consensus topologies, with bootstrap values over 90 and posterior probability values over 0.90. On this grounds, lumping *Santanderella amado-rinconiana*, and also *Macroclinium chasei* and *Macroclinium alleniorum*, or even the genera *Notylia*, *Notyliopsis*, *Macroclinium* and *Macradenia* as has been suggested as an ultimate option (F. Pupulin and M. Chase, personal communications), would seem inappropriate, specially if the studies based in morphological characters such as the one reported by Pupulin (1997) on the phylogeny of *Macroclinium* are taken into consideration. In this case, *Macroclinium chasei* appears linked only by a dotted line to the main branch of this taxonomic group. Other genera in Oncidiinae are being subjected to taxonomic transfers (Chase and Whitten, 2011), while a word of caution has been proposed on further studies of phylogenetic delimitation in plants before a world-wide consensus is reached (Vanderpoorten and Shaw, 2010).

Nevertheless, our results strongly support our hypothesis of a new genus for *Santanderella amado-rinconiana*, as an option to clarify the diversity of orchids within the Oncidiinae subgroup, both at the phenotypic and genotypic levels. We have demonstrated a clear genotypic and phenotypic separation of *Santanderella* against both *Notylia* and *Macroclinium*, further supporting the validity and specificity of *Santanderella* as based on its long branch (reflecting its clear morphological identity) compared to the other segregate genera sampled.

As stated in the introduction, establishing monotypic genera is not ideal. However, as we cannot stretch out the limits of the genera to force incongruous phenotypic elements into established genera, we also conclude that the presence of monotypic genus within this group (*Notyliopsis*, *Sarmenticola*, *Chelyorchis*, *Hintonella*, *Hofmeisterella*, *Schunkea* and *Santanderella*) implicates the existence of multiple segregate (most likely oligospecific) genera in the vicinity of the *Notylia* and *Macroclinium* "clade". The need to accept a new genus is thus based on its clear genetic differentiation from these segregate genera, but also because of its discrete and patent morphological

identity, worthy of constituting a new generic entity. As stated by some researchers (Santiago Madriñán, personal communication), this is the case of numerous examples in systematics, where speciose monophyletic groups characterized by clear autoapomorphies are accompanied by a grade of oligospecific groups –each with its own autoapomorphy–, which cannot be included in the larger groups diluting their identity as to the characters that allow their recognition, and which cannot be placed within a single entity due to their non monophyly.

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**Table 1.** Sampling of taxa to *Oncidiinae* used in this study available on local crops that were not previously reported in GenBank. Voucher numbers cited correspond to specimens with which our specimens were compared and validated.

Taxon	GenBank accession number	Source; locality	Voucher
<i>Oncidiinae</i> sp [ <i>Santanderella amaao-rinconiana</i> ]	HQ219251.1	Orlando Rincon (isotype) - Floridablanca (Santander)	P. Ortiz 1335 (HPUJ)
<i>Macroclinium xiphophorum</i>	HQ219252.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz 4358 (HPUJ)
<i>Notylia incurva</i>	HQ219253.1	Arturo José Carrillo - Villeta (Cundinamarca)	G. Misas 214b (HPUJ)
<i>Notyliopsis beatricis</i>	HQ219254.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz 1061 (HPUJ)
<i>Oncidium</i> ( <i>Trichocentrum</i> ) <i>lanceanum</i>	HQ219255.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz s.n. (HPUJ)
<i>Oncidium ornithorhynchum</i>	HQ219256.1	Roberto Carrascal - Bogotá D.C.	P. Ortiz 110 (HPUJ)
<i>Oncidium cultratum</i>	HQ219257.1	Roberto Carrascal - Bogotá D.C.	P. Ortiz 187 (HPUJ)
<i>Oncidium</i> ( <i>Otoglossum</i> ) <i>globuliferum</i>	HQ219258.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz 54 (HPUJ)
<i>Oncidium fuscatum</i>	HQ219259.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz 436 (HPUJ)
<i>Brassia</i> sp	HQ219260.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz 4210 (HPUJ)
<i>Macradenia brassavolae</i>	HQ219250.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz 895 (HPUJ)
<i>Trichocentrum pulchrum</i>	HQ219261.1	Roberto Carrascal - Bogotá D.C.	P. Ortiz 702 (HPUJ)
<i>Oliveriana ortizii</i>	HQ219262.1	Luis Eduardo Alvarez - Arcabuco (Boyacá)	P. Ortiz 101 (COL)
<i>Telipogon nervosus</i>	HQ219263.1	Luis Eduardo Álvarez - Guatavita (Cundinamarca)	P. Ortiz 970 (HPUJ)
<i>Oncidium</i> ( <i>Trichocentrum</i> ) <i>carthagenense</i>	HQ219264.1	Sócrates Forero - Silvania (Cundinamarca)	P. Ortiz 143 (HPUJ)

Table 2. Oncidiinae taxa used in the phylogenetic analysis of *matK-trnK* and *ITS1-5.8S-ITS2* sequence data. N. A.: Not available.

<u>Taxon</u>	<u>matK-trnK</u>	<u>ITS1-5.8S-ITS2</u>
<i>Macroclinium aurorae</i> Whitten 3005	FJ565118.1	FJ565626.1
<i>Macroclinium dalstroemii</i> Whitten 2509	FJ565072.1	FJ565585.1
<i>Macroclinium</i> sp. Dressler 6349.	FJ565437.1	FJ564931.1
<i>Macroclinium xiphophorum</i> isolate P. Ortiz	HQ219252.1	JN189789
<i>Macroclinium bicolor</i>	AF350629.1	AF350550.1
<i>Macroclinium robustum</i> Gerlach 93/3019 M	FJ563935.1	FJ565344.1
<i>Macroclinium alleniorum</i>	EF079188.1	EF079399.1
<i>Notylia</i> sp. Whitten 1530	FJ564966.1	FJ565482.1
<i>Notylia pittieri</i>	FJ565181.1	FJ564701.1
<i>Notylia ecuadorensis</i> Whitten	FJ565477.1	FJ564961.1
<i>Notylia</i> sp. Trujillo 427	FJ564752.1	FJ565240.1
<i>Notylia incurva</i> isolate G. Misas 214b	HQ219253.1	JN189790
<i>Santanderella amado-rinconiana</i> P. Ortiz 1335 HQ219251.1	JN189792	
<i>Notyliopsis beatricis</i> P.Ortiz 1061	HQ219254.1	JN189791
<i>Notylia</i> sp. Whitten 1544	FJ564966.1	FJ565482.1
<i>Notylia barkeri</i> Whitten 3445	FJ565300.1	AF350624.1
<i>Notylia albida</i> Whitten 2823	FJ565613.1	FJ565105.1
<i>Notylia venezuelana</i>	EF079193.1	EF079397.1
<i>Macradenia tridentata</i> Hirtz 8	FJ565405.1	FJ564896.1
<i>Macradenia rubescens</i> Gerlach	FJ564839.1	FJ565345.1
<i>Macradenia brassavolae</i> Chase O-166 K	FJ563854.1	FJ565220.1
<i>Gomesa</i> sp. Pansarin 968	FJ564919.1	FJ565426.1
<i>Maxillaria aciantha</i>	DQ209876.1	DQ210296.1
<i>Schunkea vierlingii</i> Gerlach 0-21958 M	FJ563933.1	FJ565340.1
<i>Warmingia eugenii</i> Williams N192	FJ563905.1	FJ565285.1
<i>Warmingia zamorana</i> Hirtz 7291	FJ563944.1	FJ565369.1
<i>Seegeriella pinifolia</i> Gerlach 0-22556 M	FJ564829.1	FJ565339.1
<i>Sutrina garayi</i> Gerlach 0-22308 M	FJ564828.1	FJ565338.1
<i>Ionopsis utricularioides</i> Whitten 2346	FJ565042.1	FJ565557.1
<i>Comparetia falcata</i> Whitten 2688	FJ565090.1	FJ565601.1
<i>Scelochilus</i> sp. Luis Mendoza s.n.	EF079192.1	EF079394.1
<i>Rodriguezia batemanii</i> Whitten 1615	FJ564975.1	FJ565491.1

Table 3. *matK-trnK* forward and reverse primer sequences, fragment length sequenced, and location within *matK-trnK*.

for/rev primers	sequence	length	<i>matK-trnK</i> location
390F/	CGATCTATTCAATATTTC		
1326R	TCTAGCACACGAAAGTCGAAGT	936 bp	2962-3897
19F/	CGTTCTGACCATATTGCACTATG		
556R	GAAGAAACATCTTTGATCCA	614 bp	2488-3101

Table 4. *ITS1-5.8S-ITS2* forward and reverse primer sequences, fragment length sequenced, and location within ITS.

primers	sequence	length	ITS location
17SE/	ACGAATTCATGGTCCGGTGAAGTGTTTCG		
26SE	TAGAATTCCTCCGGTTCGCTCGCCGTTAC	724 bp	18S-26S rRNA

Table 5. Characterization of DNA sequences and parsimony analyses conducted for each molecular marker used in this study.

Marker comparisons:

Marker	bp	excl. gaps	Informative sites		
			no.	% total	% excl. gaps
ITS	724	558	44	6.	7.9
<i>matK-trnK</i>	1436	1194	119	7.9	10
combined	2180	1611	163	13.9	17.9

Tree analyses:

Marker	Best tree length	# most parsimonious trees	Consistency index (excl. un-informative)	Retention index
ITS	179	3141	0.65	0.82
<i>matK-trnK</i>	412	9543	0.43	0.75
combined	749	7678	0.52	0.73



**THE ORDERS OF OSTROPOMYCETIDAE  
(LECANOROMYCETES, ASCOMYCOTA): RECOGNITION OF  
SARRAMEANALES AND TRAPELIALES WITH A REQUEST  
TO RETAIN PERTUSARIALES OVER AGYRIALES**

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**ABSTRACT**

Recent molecular phylogenetic analyses have shown that *Agyrium rufum*, the type species of *Agyrium*, is unrelated to Trapeliaceae and closely related to *Pertusaria* s. str. As such, the ordinal names Agyriales and Pertusariales were placed in synonymy, with preference given to continue the use of the older name Agyriales rather than Pertusariales. We argue that the name Pertusariales should be retained in favor of Agyriales. We also formally describe a new order, Trapeliales, to accommodate the discrete group of taxa that was previously classified in Agyriales but is actually distinct from that taxon in its current circumscription. Additionally, the molecularly and morphologically distinct order Sarrameanales is defined. *Phytologia* 93(3):407-412 (December 1, 2011)

**KEY WORDS:** Fungi, lichen, *Loxospora*, Loxosporales, nomenclature, *Sarrameana*, Sarrameanaceae, taxonomy

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Recently the authors began updating the synoptic higher level taxonomic framework of lichenized fungi that is used to organize the lichen herbarium of the New York Botanical Garden (NY; Hodkinson, in press). Such updates are intended to keep the herbarium organized in a phylogenetic framework that reflects current taxonomic concepts.

However, they also offer useful opportunities to compare and synthesize the accumulated results of recent studies based on both molecular and non-molecular datasets. While preparing the current scheme, we could not find a source of valid publication for an ordinal name encompassing the family Sarrameanaceae Hafellner (= *Loxosporaceae* Kalb & Staiger), although “*Loxosporales?*” was used by Miadlikowska et al. (2006). Since doubt was expressed, this does not constitute valid publication, and we could not find any definitive use of an ordinal name in subsequent publications. However, members of the genus *Loxospora* (contained within Sarrameanaceae) are well supported by several large-scale phylogenetic analyses as being distinct and separate from the clade containing all other defined orders within Ostropomycetidae (Miadlikowska et al. 2006; Schoch et al. 2009) and therefore this clade warrants recognition at the ordinal rank.

In our review we also found that Lumbsch et al. (2007a) reduced Agyriales to the type family (Agyriaceae) and genus (*Agyrium*) based on the fact that *Agyrium rufum* (Pers.) Fr. (the type species of the *Agyrium*) did not show phylogenetic affinities to Trapeliaceae, a family comprising all of the other genera previously included in the order. In the same work, Trapeliaceae was moved to the order Baeomycetales, based on analyses presented by the authors showing a well-supported sister relationship between Trapeliaceae and a clade correlated with Baeomycetales.<sup>1</sup> The sister relationship that was inferred stands in contrast to some analyses which place Trapeliaceae in a position where it is strongly supported by Bayesian inference as neither being sister to nor within Baeomycetales (e.g., Kauff & Lutzoni 2002; Lutzoni et al. 2004; Miadlikowska et al. 2006). Regardless of which reconstruction best reflects the evolutionary history of the organisms, all of the analyses for which we have examined the results put members of Trapeliaceae in a clade that is molecularly and morphologically distinct from Baeomycetales. In light of the current situation, we formally establish Trapeliales as a new order to accommodate the family Trapeliaceae. The genera of Trapeliaceae that form the core of the

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<sup>1</sup> Note that the genus *Ainoa* Lumbsch & I. Schmitt, which is often treated with Trapeliaceae, occupies an uncertain taxonomic position, but is inferred to be closely affiliated with Baeomycetales (Lumbsch et al. 2007a, 2007b; Schmitt et al. 2010).

family in large-scale molecular phylogenetic studies are *Placopsis* (Nyl.) Linds., *Placynthiella* Elenk., *Orceolina* Hertel, *Rimularia* Nyl., *Trapelia* M. Choisy, and *Trapeliopsis* Hertel & Gotth. Schneid. (Schmitt et al. 2003; Lumbsch et al. 2007a, 2007b, Miadlikowska et al. 2006), while the genera *Aspiciliopsis* (Müll. Arg.) M. Choisy, *Lignoscripta* B.D. Ryan, *Lithographa* Nyl., *Ptychographa* Nyl. and *Xylographa* (Fr.) Fr. have been shown otherwise to have molecular affinities to the core clade of the family (Spribille, pers. comm.; Lumbsch et al. 2001; Schmitt et al. 2003); additionally, the genera *Amylora* Rambold, *Coppinsia* Lumbsch & Heibel and *Sarea* Fr.<sup>2</sup> are provisionally placed here based on morphology.

Another work that recently came to our attention was Schmitt et al.'s (2010) paper on *Gyalectaria*, in which *Agyriales* was put into synonymy with *Pertusariales*. The Schmitt et al. (2010) publication revealed a close relationship between *Agyrium rufum* (Pers.) Fr. and *Pertusaria* DC. s. str. (typified by *P. communis* DC. = *P. pertusa* (L.) Tuck.). This relationship is surprising because *A. rufum* is a non-lichenized saprophytic fungus that does not morphologically resemble *Pertusaria* s. str. Nonetheless, the relationship was well supported by both maximum likelihood and Bayesian methods of phylogenetic inference, and based on sequence data from four genes and two samples. *Pertusaria* DC. is the type genus of the family *Pertusariaceae* Körb., which is the type of the order *Pertusariales* M. Choisy ex D. Hawksw. As a consequence of the recognition that *A. rufum* is strongly supported as sister to members of *Pertusaria* s. str., Schmitt et al. (2010) concluded that the names *Agyriales* and *Pertusariales* should be placed in synonymy. When Schmitt et al. (2010) placed the two orders in synonymy, they adopted the name *Agyriales* for this group on the basis of priority. However, the principal of priority does not apply above the rank of family and, thus, either name could have been adopted (McNeill et al. 2006: Art. 11.10). It is recommended that, when working with names above the rank of family, authors should follow priority (McNeill et al. 2006: Rec. 16B); however, we believe that this

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<sup>2</sup> Although sequences generated by Reeb et al. (2004) indicate that *Sarea* does not belong to *Ostropomycetidae*, and may be closer to clades currently placed in *Leotiomyces*, this change is not reflected in the current outline (Lumbsch & Huhndorf 2010); since these sequences could potentially be derived from contaminants, we leave the genus in *Trapeliaceae* provisionally.



situation presents a clear case in which the name Pertusariales should be retained. The reason for this is that Pertusariales, excluding *Agyrium*, comprises a group containing hundreds of species of lichenized fungi that, for the most part, have historically been grouped together because of their distinctive morphological gestalt. Conversely, the name Agyriales has previously been applied to an incorrectly circumscribed group including the family Trapeliaceae, and was originally applied to a large heterogeneous group of organisms from across the fungal tree of life. In a strict sense, the name Agyriales applies to a small genus of less than two dozen species of non-lichenized saprophytic fungi, only one of which, the type, has been sequenced to date and included in molecular phylogenetic analyses (Schmitt et al. 2010). We assert that the name Pertusariales should be retained in the interest of stability to preserve the continued use of a name for a highly speciose group that has become widespread and has proliferated throughout the lichenological literature.

### TAXONOMIC SECTION

Note: The lower-level composition of each taxon is given only if it differs from that shown by Lumbsch & Huhndorf (2010).

Ostropomycetidae Reeb, Lutzoni & Cl. Roux

Baeomycetales Lumbsch, Huhndorf & Lutzoni

Anamylopsoraceae Lumbsch & Lunke

Baeomycetaceae Dumort.

Ostropales Nannf.

Pertusariales M. Choisy *ex* D. Hawksw. & O.E. Erikss.

= Agyriales Clem. & Shear

Agyriaceae Corda

Coccotremataceae Henssen *ex* J.C. David & D. Hawksw.

Icmadophilaceae Triebel

Megasporaceae Lumbsch, Feige & K. Schmitz

Ochrolechiaceae R.C. Harris *ex* Lumbsch & I. Schmitt

Pertusariaceae Körb. *ex* Körb.

Sarrameanales Hodkinson & Lendemer ord. nov.

Sarrameanaceae Hafellner [Type family]

= Loxosporaceae Kalb & Staiger

Trapeliales Hodkinson & Lendemer ord. nov.

Trapeliaceae M. Choisy *ex* Hertel [Type family]



Families incertae sedis

Arctomiaceae Th. Fr.

?Arthrorhaphidaceae Poelt & Hafellner

Hymeneliaceae Körb.

Protothelenellaceae Vězda, H. Mayrhofer & Poelt

Schaereriaceae Hafellner

Thelenellaceae H. Mayrhofer

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# INDEX TO ARTICLE TOPICS AND SCIENTIFIC NAMES IN VOLUME 93

(New scientific names and combinations printed in bold face.)

<i>Abies concolor</i> .....	107, 208, 221
<i>Abies lasiocarpa</i> .....	73
<b>Ageratina tovarae</b> .....	94
ALOYSIA Palau (a correction).....	388
<i>Blyttiomycetes</i> .....	304
<i>Blyttiomycetes spinulosus</i> .....	305
<b>Brickellia enigmatica</b> .....	322
<i>Brickellia grandiflora</i> (distribution).....	328
<i>Brickellia odontophylla</i> (distribution).....	327
<i>Brickellia simplex</i> (distribution).....	327
<i>Dalea nana</i> .....	181
<i>Dalea reverchonii</i> .....	181
<i>Decachaeta incompta</i> .....	346
<b>Decachaeta serboana</b> .....	346
<i>Fraxinus americana</i> .....	70
<i>Fraxinus caroliniana</i> .....	70
<i>Fraxinus caroliniana</i> var. <b>pauciflora</b> .....	71
<i>Fraxinus profunda</i> .....	71
<i>Hedeoma drummondii</i> .....	174
<i>Hedeoma reverchonii</i> .....	174
<i>Iris brevicaulis</i> .....	231
<i>Iris fulva</i> .....	238
<i>Iris germanica</i> .....	240
<i>Iris hexagona</i> .....	239
<i>Iris savannarum</i> .....	231
<i>Iris savannarum</i> var. <b>kimballiae</b> .....	236
<i>Iris tridentata</i> .....	238
<i>Iris virginica</i> .....	238
<i>Jaltomata atiquipa</i> .....	203
Juniper and Spinach leaves storage.....	283
<i>Juniperus arizonica</i> .....	43
<i>Juniperus blancoi</i> .....	3, 132, 168
<i>Juniperus californica</i> .....	245
<i>Juniperus chinensis</i> .....	118
<i>Juniperus communis</i> .....	185

<i>Juniperus excelsa</i> .....	316
<i>Juniperus pinchotii</i> .....	146
<i>Juniperus polycarpos</i> var. <i>polycarpos</i> .....	316
<i>Juniperus tsukusiensis</i> var. <b><i>taiwanensis</i></b> .....	118
<i>Juniperus maritima</i> .....	3
<i>Juniperus scopulorum</i> .....	3, 132, 168
<i>Juniperus virginiana</i> .....	3, 51, 168
<i>Juniperus virginiana</i> , DNA degradation.....	351
<i>Lagena</i> .....	157
<i>Lagenidium</i> .....	157
<i>Lagenocystus</i> .....	157
<i>Lophophora williamsii</i> .....	330
<i>Loxospora</i> .....	407
<i>Malacomeles denticulata</i> .....	100
<i>Malacomeles nervosa</i> .....	101
<i>Malacomeles paniculata</i> .....	101
<b><i>Malacomeles pringlei</i></b> .....	102
<b><i>Malacomeles psilantha</i></b> .....	102
<i>Oncidiinae</i> .....	388
<i>Orchidaceae</i> .....	388
<i>Phacelia marshall-johnstonii</i> var. <b><i>deliciasana</i></b> .....	88
<i>Physalis angustifolia</i> .....	260
<i>Physalis cinerascens</i> .....	260
<i>Physalis cinerascens</i> var. <b><i>variovestita</i></b> .....	263
<i>Physalis mollis</i> .....	260
<b><i>Physalis spathulifolia</i></b> .....	260
<i>Physalis viscosa</i> .....	260
<i>Physalis walteri</i> .....	260
Pinyon-Juniper forest in Nevada.....	360
<i>Pythium</i> .....	157
<b>Sarrameanales</b> .....	407
<b><i>Scutellaria serboana</i></b> .....	241
<i>Senecio parryi</i> .....	341
<i>Senecio pringlei</i> .....	342
<i>Senecio ritovegana</i> .....	342
<i>Santanderella amado-rinconiana</i> .....	388
Spermacoce of Florida.....	275
<i>Spermacoce assurgens</i> .....	280
<i>Spermacoce confusa</i> .....	281



Spermacoce densiflora.....	279
Spermacoce glabra.....	280
Spermacoce ocymoides.....	279
Spermacoce tenella.....	281
Spermacoce tenuior.....	281
Spermacoce tetraquetra.....	281
Spermacoce verticellata.....	280
Styrax platanifolius.....	198

### Correction

Recently (Phytologia 92(2): 199-205, 2010 ) the title for the paper "SUMMARY OF LECTOTYPES ASSOCIATED WITH *ALOYSIA PALAU* (VERBENACEAE)" was inadvertently published with the genus and author both italicized (eg. *ALOYSIA PALAU*). To avoid any confusion, the title should have been: SUMMARY OF LECTOTYPES ASSOCIATED WITH *ALOYSIA* PALAU (VERBENACEAE).

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